The principle of safety evaluation in medicinal drug - how can toxicology contribute to drug discovery and development as a multidisciplinary science? -

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ABSTRACT — Pharmaceutical (drug) safety assessment covers a diverse science-field in the drug discovery and development including the post-approval and post-marketing phases in order to evaluate safety and risk management. The principle in toxicological science is to be placed on both of pure and applied sciences that are derived from past/present scientific knowledge and coming new science and technology. In general, adverse drug reactions are presented as “biological responses to foreign substances.” This is the basic concept of thinking about the manifestation of adverse drug reactions. Whether or not toxic expressions are extensions of the pharmacological effect, adverse drug reactions as seen from molecular targets are captured in the category of “on-target” or “off-target”, and are normally expressed as a biological defense reaction. Accordingly, reactions induced by pharmaceuticals can be broadly said to be defensive reactions. Recent molecular biological conception is in line with the new, remarkable scientific and technological developments in the medical and pharmaceutical areas, and the viewpoints in the field of toxicology have shown that they are approaching toward the same direction as well. This paper refers to the basic concept of pharmaceutical toxicology, the differences for safety assessment in each stage of drug discovery and development, regulatory submission, and the concept of scientific considerations for risk assessment and management from the viewpoint of “how can multidisciplinary toxicology contribute to innovative drug discovery and development?” And also realistic translational research from preclinical to clinical application is required to have a significant risk management in post market by utilizing whole scientific data derived from basic and applied scientific research works. In addition, the significance for employing the systems toxicology based on AOP (Adverse Outcome Pathway) analysis is introduced, and coming challenges on precision medicine are to be addressed for the new aspect of efficacy and safety evaluation.

Key words: Evolutional toxicology, Molecular toxicology, Systems toxicology, Adverse outcome pathway, Precision medicine, Risk assessment/management

INTRODUCTION

Until now, safety assessment as part of the pharmaceutical development process has mainly focused on toxicity studies necessary for the approval of INDs (Investigational New Drugs) and NDAs (New Drug Applications). Namely, the main purpose has evolved to be used for evaluation and approval when submitting new drug applications and safety assessment of drugs used for EIH (Entry-In Human), Phase 1 clinical trials and final approval for the marketing. Safety assessment has been necessary for selection of clinical candidate compounds to optimize lead compounds in the early stage of drug discovery, and recently a systematic approach for high-throughput toxicology (HTP-Tox) study is being established to fulfill these demands. Toxicology studies necessary at each step from drug discovery to clinical development and post-marketing are classified as follows: 1) toxicology studies
for screening and the early stages of drug development, 2) exploratory toxicology studies to select clinical candidate compounds, 3) toxicology studies for safety assessment of initial clinical studies (EIH, IND) in humans, 4) toxicology studies for NDAs, and 5) toxicology studies for confirmation of post-marketing safety assessment. However, the procedures of study methods and assessment are different in each stage and their approaches are case-by-case in actuality depends on each stage of drug discovery and development.

Recently, disease-specific molecular biology has evolved due to an etiological elucidation based on the newest molecular biology for drug discovery. Toxicology in safety assessment for drug discovery & development must keep up with major changes in the progress made in science and techniques for medical drugs. Investigation based on multidisciplinary sciences is necessary for precise drug safety assessment for all stages of drug discovery and development. It is also necessary not just to simply show the appearance of toxicities but also to elucidate the mechanism of expression of the toxicities to move towards both predicting and managing with their toxicities. Thus, the adoption of new and multidisciplinary scientific techniques is essential, and the molecular toxicological approach and systems toxicology are moving towards more important positions.

The biological changes in the toxicological expression processes from destruction and development to repair are diverse and complicate. Generally, it is necessary to elucidate the mechanism of toxic expression with various scientific aspects of safety assessment which include toxicology, pharmacology, pathology, pharmacokinetics, biochemistry, biology, physiology, molecular biology, physical chemistry, etc. In addition to toxicology assessment based on multidisciplinary sciences, the molecular toxicological approach, which makes use of the newest scientific technology such as toxicogenomics to analyze the manifestation of toxicity from the aspects of gene expression, is advancing.

During the course of drug discovery and development, toxicologist as a party in charge is always facing to the go/no-go decision from early drug discovery to clinical trials, application for approval, and post marketing processes. The axis of this decision in risk assessment and evaluation bases on whether there are means to avoid adverse drug reactions, whether non-clinical studies can be extrapolated to adverse drug reactions in humans, and if there is a beneficial balance for patients between the efficacy and the adverse reactions. Looking toward the future, the contribution of the general and basic toxicology field is essential to applied science, which occupies the gap between regulatory science and pure basic science.

**BASIC CONSIDERATIONS ON THE EXPRESSION OF DRUG-INDUCED TOXICITY**

**The relationship between medicinal efficacy and side effects (Fig. 1, Fig. 2)**

The relationship between medicinal efficacy and toxicity has been noted since long ago. Paracelsus (1493-1541), known as the father of toxicology, has proposed a fundamental idea, “All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy.” (Klaassen, 2008) Viewed from the standpoint of pharmaceuticals, the “medicine and poison” relation could be described by saying, “All medicines are poisons, there is nothing that is not poison, and it is only the dose that makes the difference between poison and medicine.” It is common knowledge that medicinal efficacy and toxicity are opposite sides of a two-edged sword, and that, to the extent that there is medicinal efficacy, there will also be adverse reactions.

It is possible to understand one aspect of the dual medicinal and toxicity of pharmaceuticals by taking the concepts of evolutionary medical science and evolutionary toxicology (Horii, 2010) (Fig. 1). It has become clear that many symptoms or signs thought to appear from “sicknesses” are in fact defense reactions that serve to protect the living body, and even that the survival of the human race has actually been assisted by genes that had been thought to induce illness. This thought called “evolutionary medicine”, was proposed in the 1990s by Randolph Nesse and George Williams (Nesse and Williams, 1995). Conventional medical science asks questions such as “What makes people sick?” and “How do people become sick?” (Lappe, 1994). It ultimately attempts to identify the genes that cause sickness and their expression patterns. However, there has been little work done from the approach of “Why do people become sick?” (Imura, 2006, 2008). It is said that all living things are the products of the evolutionary process operating via natural selection, which largely depends on the direction is set by the comparative relationship between gene mutations that can become the cause of a disease and alleles that confer resistance to that disease. In any case, the proposition that most diseases are caused by the interaction of genetic and environmental factors connects as a logical extension of Darwin’s theory of evolution (Darwin, 1859). Viewed this way from the standpoint of evolutionary medical science, medicinal efficacy that enables a response to diseases involving organismal regulation and maintenance can be thought of as that which displays
the supportive action of redirecting diseased states due to functional or structural abnormalities to the path toward normalcy and curing. However, for an organism, pharmaceuticals (drugs) are foreign substances whose toxicity presents as “biological reactions to foreign substances.” Considered scientifically in this way, toxicity can be thought of as harmful adverse reactions in the category of expressions of toxicity induced by targets that are extensions of a pharmaceutical’s medical efficacy or not. Expression of toxicity is a responsive action to a foreign substance (pharmaceutical), and the series of “an organism’s defense reactions to a foreign substance,” understood as organisms → tissues → cells → intracellular organelles, traces a path from all maintenance of normal functions to expression of damage (functional or structural damage). This can also be said to be the expression of “a trigger or additive/accelerative effects for the abnormal condition” in the sequence of a biological response in the process from normal conditions to abnormal conditions. Basically expressed as defense reactions of the organism, adverse reactions induced by pharmaceuticals are, in a broad sense, defense reactions.

Safety assessments of toxicity from the standpoint of evolutionary toxicology probably assist risk assessment and risk management of whether the toxicity is critical or not, or manageable or not. Conventional toxicology has focused on investigating questions such as “What causes the toxicity?” and “How does the condition of the toxicity arise?” and has attempted to clarify any causative mechanisms by introducing new scientific disciplines such as molecular toxicology. However, there has not been enough work done in the approach from a fundamental perspective, such as “Why does the toxicity arise due to exposure to the compound (pharmaceutical)?” This kind of evolutionary toxicological consideration will supply researchers with important hints as we come to understand how a pharmaceutical acts as a trigger for the expression of toxicity and how it exerts influence on the induction of toxicity (Bickham and Smolrn, 1994). This approach will probably set the course for making precise risk assessment and risk management with respect to the induction of adverse reactions seen in drug therapy.

**Pharmacological efficacy and toxicity in target cells (Fig. 2)**

From the perspective of target cells involved in pharmacological efficacy and toxicity, the intra- and extracellular transition-modality (absorption, distribution, reaction, metabolism, and excretion) of each compound are involved in the induction of various cellular reactions. The targets are intracellular organelles (nucleolus, nucleus, ribosomes, follicles, rough endoplasmic reticulum, Golgi body, microtubules, smooth endoplasmic reticulum, mitochondria, vacuoles, cytoplasmic matrix, lysosomes, centrosomes, etc.). Although the pharmacological and toxicological effects exhibit multiplicity, their characteristics and strength depend on the intracellular exposure levels and retention time of the compounds. A com-
compound’s intracellular absorptive, metabolic, and excretive functions (transporters, drug-metabolizing enzymes, etc.) are involved in the exposure levels and retentions times, and they affect pharmaceutical efficacy and toxicity. The intracellular (and intranuclear) reactions caused by a compound and its metabolites at target cells are induced as molecular biological and molecular toxicological reactions in organelles, and DNA, RNA, mRNA, tRNA, rRNA, miRNA, siRNA, proteins, and other products interact in complex ways during exposure to the compound in the regulation and maintenance of cellular functions, and they present as pharmacological action or toxicity (Zimmer, 2007).

**Multidisciplinary science evaluation of toxicity**

From long ago, toxicology has been designated as research dealing with adverse effects of foreign substances. This research field is based multidisciplinary science, and unavoidably drew upon approaches from diverse science fields. In order to accurately carry out safety evaluations, estimation procedures are sought from diverse scientific fields. If one looks at the related scientific systems needed for detection, analysis, and evaluation of the toxicity currently confronting the field, the elucidation of the mechanisms of toxicity requires a comprehensive safety evaluation that draws on scientific viewpoints from diverse scientific fields including toxicology, pharmacology, pathology, pharmacokinetics, biochemistry, biology, physiology, molecular biology, and physical chemistry, etc. In recent years, toxicology has outgrown the research category of investigating the adverse effects of extrinsic substances and has begun investigating changes that occur in response to toxicants from the perspective of molecular biology. That is, in addition to multidisciplinary based evaluations of toxicity, there are advances being made in the multifaceted analysis of the mechanisms of toxicity expression analysis from a molecular-toxicological approach using new scientific technology such as toxicogenomics. Perspectives of strict risk assessment and prudent risk management are also needed to understand toxicity. The trend of these analyses (systems toxicology, pathway analysis based on AOP) by interpreting them as systems biology (the comprehensive and multidisciplinary scientific investigation) is continuing to take a position of increasing importance. It is no exaggeration to say that the expression toxicity of pharmaceuticals also falls within this category.

The expression of toxicity is generally understood as progressing through the following four steps. Expressed collectively, they are 1) the exposure to the toxicant (transition to the exposure or target site: absorption, distribution, excretion, re- absorption, detoxification, poisoning),
Principle of safety evaluation in medicinal drug

MECHANISM OF TOXICITY EXPRESSION (FIG. 3)

Multidisciplinary scientific perspectives on toxicity onset

In risk assessment and management in pharmaceutical safety evaluations, it is important to elucidate the mechanism of toxicity based on all data from the various academic fields listed below (Nelson and Pearson, 1990; Bursch et al., 1992; Boelsterli, 1993; Buja et al., 1993; Baumann and Gauldie, 1994; Raghow et al., 1994; Hardman et al., 1995; Cohen et al., 1997; Wyllie, 1997; Darzynkiewicz et al., 1998; Kroemer et al., 1998; Toivala and Eriksson, 1999; Daniel, 2000; Wallace and Starkov, 2000; Blatt and Glick, 2001; Williams, 2001; Boatright and Salvesen, 2003; Roth et al., 2003; McGowan and Russell, 2004; Meijer and Codogno, 2004; Moggs and Orphanides, 2004; Orrenius, 2004; Cribb et al., 2005; Mehendale, 2005; Rhee et al., 2005; Boelsterli, 2007; Horii, 2010).

2) the reaction between the toxicant and the target molecule (mode of action: covalent bond, dehydrogenation, electron transfer, and enzyme reaction; reaction to the target molecule: dysfunction, destruction, and immunization), 3) the toxicant-induced toxicity and impairment of cellular function (cell dysfunction: gene expression-control disorders, and cellular activity control disorders; toxic changes for cell maintenance: impairment of intracellular maintenance, impairment of extracellular function maintenance), and 4) repair and repair disorders (molecular repair: protein, lipid, and DNA repair; cell and tissue repair: apoptosis, proliferation, necrosis, fibrosis, carcinogenic effects (Klaassen, 2008). From the viewpoints of the various science fields stated above, understanding of any mechanisms of expression of toxicity means that incidents in which the organism ultimately recognizes an exogenous pharmaceutical as a foreign substance is observed as a functional or structural change, and that incident acts as adverse effects additively and acceleratively on the processes of maintenance and regeneration, division and proliferation, degradation and destruction, whereby the normal organism (intracellular organelles, cells, tissues, and organs) eventually regains homeostasis. Expression of the modes of actions present as vasodilatation and hemorrhage, thrombosis and infarction, and deposition and accumulation for changes involving maintenance of homeostasis and regeneration; as degeneration, necrosis, apoptosis, phagocytosis, and organization for changes involving degradation and destruction; and as carcinogenicity (tumorigenesis), teratogenicity (developmental abnormality), increased cell division (hyperplasia) for changes involving division and proliferation. It is important to use such ways of evaluation as a basis when interpreting the results of toxicity studies and validating those evaluations from the perspectives of the identification of toxicity, pharmacological efficacy and toxicity, and exposure levels and toxicity.

Cytological perspective on toxicity manifestations

Toxicological intensity depends largely upon the relevant intracellular exposure level and retention time of the compound. The exposure pattern is determined by the following cell functions and drug actions within the relevant cell: the retention mode of the compound in the cell (absorption systems through the membrane: transporters; involvement of the vascular system: blood circulation) → the mode of existence within the cell (involvement of drug metabolizing enzymes) → the mode of excretion out of the cell (excretion systems through the membrane: transporters; involvement of the excretory system: excretion to blood, bile, and urine). The manifestation mode of toxicity is expressed as medicinal efficacy when metabolites and the unchanged compound act on the pharmacological and toxicological intracellular target receptor, and its toxicity induced by excess reactions or interactions with various intracellular functional substances (DNA, RNA, mRNA, tRNA, rRNA, miRNA, siRNA, protein, lipid, carbohydrates, etc.) is manifested as an expression system. Toxicity involving this type of cellular regulation and maintenance present in the cell as functional and structural disorders.

Pharmacological perspective on toxicity manifestations

In pharmacology, toxicity can be understood as the reactions to a foreign substance at the receptor or gene level, induced by the drug transition state to cells and tissues. Various target cells undergo gene regulation during the process from uptake to clearance of a foreign substance in an organism (absorption, distribution, metabolism, and excretion). Chemical substances taken up into the cell, acting via gene regulatory actions, induce target molecule production (enzymes and regulatory substances) and reactions at the receptor level, and are expressed as pharmacological actions. When these actions are excessive, they are expressed as toxicity when they act as toxic disorder reactions and result in malfunction or breakdown of architecture of cells. Followed by these reactions, additional disorders occur secondarily as a restorative cellular and biological reactions are evoked to recover those disorders.

By using pharmacokinetics analysis of the compound,
this series of reactions can be understood from the aspects of absorption, distribution, metabolism, and excretion, and can be understood from exposure conditions (concentration and retention time) at the disorder site and target site. In addition, by investigating the reactions between the target molecule and effectors, the medicinal efficacy and toxicity, as part of the biological defense mechanism, are defined pharmacologically and toxicologically as functional disorders, resistance, cell breakdown, and repair.

Physiological and pathological perspectives on toxicity manifestations
Following recognition of the foreign substance occurring as physiological and histopathological reactions to external stimulation of the organism or foreign substances (pharmaceuticals), functional changes, endocrine reactions, histological changes, etc., are induced. Infiltration and migration of white blood cells, phagocytosis of foreign substances, antibody production, etc., are seen as initial reactions that begin with foreign substance recognition, and then, changes injurious to tissues are induced (perivascular or vascular lesions, encapsulation, cell degeneration, etc.). In addition, happening in parallel to initial recognition of the foreign substance, vasoconstriction, vasodilation, and hyperemia are observed as physiological function reactions and endocrine reactions followed by proliferative and degenerative changes to the cell. If these changes progress to structural changes, they may develop via gene disorders into degeneration, necrosis, abnormal proliferation, malformation, and tumorogenesis. For each of these changes, restorative changes toward the normal state occur in parallel, and in the abnormal regions, phagocytosis of degeneration and necrosis products, and tissue repair patterns from reparative reinforcement begin to appear.

Biochemical perspective on toxicity manifestations
When toxicity is understood as a biochemical reaction on toxicity-related effectors, a molecular-biological approach usually becomes necessary for clarifying gene expression involved in initial actions at the target site of the toxicity, biological defense, resistance, and repair actions, and activation for cellular breakdown. Whether

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**Fig. 3.** Onset/regulation of toxicological effects in target cell function.
the target site exhibits organ specificity or non-specificity is investigated, and the parameters involved in the mechanisms of toxicity manifestation such as reactions of compounds on effectors and the resulting biological reactions are understood from a molecule toxicological perspective based on biochemical findings; this develops into systems toxicology based on pathway analysis of that reaction, and can direct researchers in establishing key toxicological biomarkers.

Practical clues in the elucidation of mechanism of toxicity; reactions on target effectors and its biological reactions

In elucidating the mechanism of toxicity, necessary approaches include first grasping the movement of the relevant compound within the organism. Using the results of each type of toxicity study to accurately make explicit what the main toxicity is, and then revealing the mechanism of toxicity manifestation from the presentation of a hypothesis based on investigational toxicity studies and a review of published literature on the subject and the results of those validation experiments.

Pharmacokinetic and toxicokinetic investment in the organism and its pharmacodynamic effects

In drug development, it is necessary to grasp the movement of the compound within the organism and its pharmacodynamic and pharmacological effects. Evaluation of drug safety using both factors for PK/TK (pharmacokinetics/toxicokinetics) and PD/TD (pharmacodynamics/toxicodynamics) is indispensable. To know the movement modality of the compound within the organism, an investigation is made into the TK factors (the toxicological dynamics of the compound within the organism) relating to the question “How is the compound incorporated at target sites in the organism (organs, tissues, and cells), metabolized and distributed?” (Sugiyama et al., 1996; Horii, 1999; Lin et al., 2003; Ekins et al., 2005). In addition, the TD factors (the toxicological movement of the compound within the organism) relating to the question “How does the compound act on the primary-target-molecule (including the secondary-signaling-pathway and the effector-mechanism)?” are estimated, and the modality of the toxicity understood from the exposure condition is investigated. Statin (Cerivastatin) is an example of toxicity manifestation caused by TK factors. Induction of cardiotoxicity (increase of exposure level and prolongation of retention time in the heart) occurs due to drug competition and additive toxicity caused by a decrease in the Cerivastatin excretion rate and an increase in systemic exposure due to two factors: biotransforming enzyme (CYP3A4) and canalicular-export-pump (MDR1). Thalidomide is an example of toxicity manifestation caused by TD factors. Its toxicity manifestation is due to binding of the compound to a toxicity-related target gene (thalidomide binds specifically to a promoter gene involved with a growth-factor that is an integrin production factor, and induces teratogenic actions) and exposure level and exposure period are highly involved in its manifestation modality.

The challenge of elucidating the mechanism of toxicity manifestation: Reactions of compounds on effectors and the resulting biological reactions related to toxicity

Toxicological reactions of the organs and cells to the compound are varied, and changes are depending on the compound’s physical properties, exposure level and retention state and its time. These reactions are expressed as stress reactions, adaptive responses, and breakdown and repair reactions. Toxicity presents as functional disorders, endocrine disorders, inflammatory reactions, immune response disorders, metabolic disorders, gene disorders, and cellular breakdown and repair reactions. To know toxicity appearance in respective organs, it is important to confirm whether that toxicity exhibits organ specificity or non-specificity. Regarding this organ specificity, it is often the case that the changes, rather than occurring in a single organ, extend into multiple organs, and the seriousness of the toxicity also differs depending on the target organ. This depends on the variety of cellular sensitivity that responds to the function in each organ and tissue. In the elucidation of the mechanism of toxicity manifestation, confirmation of the type of major toxicity is important.

The following is type of major toxicities.

- Transport within tissues and selective accumulation
- Biological activation into active metabolites
- Oxidative stress: cellular damage, information transfer, gene regulation
- Disturbance and destruction of intracellular calcium homeostasis
- Necrotic and apoptotic cell death
- Dysfunction in cell growth and tissue repair
- Covalent bonding of active metabolites with the cellular polymers
- Immunological dysfunction
- Cytokine-related disorders
- Specific inactivation of enzymes and other proteins
- Nuclear receptor-related disorders
- Interaction with transporters
- Cellular energy production disorders
- Individual gene expression disorders
Each of these reaction modes is induced independently, concurrently, or consecutively as the case, and each is expressed as a toxicity that displays various modalities.

**MOLECULAR TOXICOLOGICAL APPROACH**

**Molecular toxicology based on molecular biology**

Toxicology is a multidisciplinary with a variety of phenomenological processes from toxicity onset to development and reversibility. Considering the relevant scientific disciplines that are required for the analysis, detection, and evaluation of toxicity, it is considered necessary to conduct safety assessment that is inclusive of the scientific perspective of various fields such as toxicology, pharmacology, pathology, pharmaco/toxicokinetics, biochemistry, biology, physiology, molecular biology, etc. to elucidate the mechanism of the toxicity. The origin of the expression of the toxicity lies on gene expression. Significant evaluations of toxicity are achieved through the multi-faceted analysis of the toxicity expression mechanism and a molecular toxicological approach using the most recent science and technology. The need to comprehend toxicity for stringent risk assessment and sensible risk management has increased. Systems biology (systems toxicology) is a comprehensive scientific elaboration stemming from this multidisciplinary science and has come to occupy an increasingly important position.

The practice of pharmaceutical drug development will become subjected to molecular targeting from the perspective of pharmacogenomics, and in parallel with the introduction of new technologies such as combinational chemistry and biologics, early stages of drug discovery and development will call not only for pharmaceutical screening but also for toxicological investigations. A variety of experiments have been introduced to address the need for the development and introduction of new in vitro/in vivo toxicity screening systems which have been created in response to “High Throughput Toxicology (HTP-Tox)”. In recent years, toxicity evaluation as a molecular toxicological approach has come under close scrutiny from the point of view of omics via toxicopanomics (toxicogenomics, toxicoproteomics, and toxicometabonomics) (Waring and Ulrich, 2000; Pognan, 2004). In other words, the omics approach, in parallel with the pharmacogenomics approach, combines HTP-Tox with Toxicopanomics to comprehend the manifestation of toxicity from the concept that “Toxicity is the result of the expression of related genes” (Hirabayashi and Inoue, 2008). Metabonomics, which is the next stage of toxicogenomics/toxicoproteomics, (Genome→Transcriptome→Proteome), is being introduced in toxicity evaluation, and parameters which show changes in a variety of molecular toxicological research are used as new endpoints for toxicological safety assessment. These omics will expand into clinical fields.

Additionally, elements for in vivo exposure (absorption, distribution, metabolism, and excretion) are desirable. The investigation of the involvement of P450 and transporter in toxicity, and molecular pharmacokinetic approach with a further Toxico-DMPK as well as toxicokinetics has been developed by fusion with molecular toxicology.

**Toxicological biomarkers and systems toxicology**

It is important to elucidate the origin of toxicity and to select appropriate markers for safety assessment. There is a variety of perspectives in drug development, and it is necessary to consider selecting toxicologically significant biomarkers from the following different points of view: “Should the trigger of toxicity manifestation be investigated? Should the major target of the toxicity be identified? Should the outcome of the manifestation be verified?” Attempting to verify biomarkers from the results of the toxicity study is no different from identifying the charred remains of a fire; they are not significant “real-time” biomarkers. It is vital that a toxicologically significant biomarker should be set during safety assessment from the early stages of drug discovery. Conventional biomarkers have been used routinely used in clinical examination and histochemistry, but in recent years human gene analysis has advanced rapidly and molecular toxicological approaches with genetics have grown in number along with the request of elevated safety assessment in drug development (Orr, 2006).

After pathway analysis by molecular toxicology, deployment of systems toxicology with key biomarkers becomes the bridge to the elucidation of toxicity manifestation. A step-by-step molecular profiling is required from early phase of drug discovery until clinical trial/approval/marketing. Data from genomic expression, proteomics, and metabolomics can be used for profiling. The basics of systems toxicology (integrative data mining, pathway analysis, biomarker identification, hypothesis generation) in each test phase (in vitro study in early drug discovery, in vivo pre- or non-clinical studies, and clinical trials) are used not only as “the platform at each stage” but also “across the platform”. To otherwise predict toxicity, toxicity-related data are accumulated with commercial database, and the fusion of selection and development of the lead compound from the correlation of the structure and
toxicity leads to the introduction of computer system with a structured database for predicting toxicity is underway, with the goal of predicting the toxicity of compounds in early drug discovery. These actions lead to the establishment of biomarkers based on pathway analysis. The concept of systems biology has become necessary for the comprehensive investigation of such drug discovery and development (Waters and Fostel, 2004; Waters and Yauk, 2007).

The next generation of molecular toxicological investigation (Non-coding RNA and epigenetic toxicology) (Fig. 4)

The model for the DNA structure was presented by Watson and Crick in 1953, and its biological significance led to successive revelations of gene expression. Gene expression has been understood with a focus on DNA as a sequence of genomics → proteomics → phenomics and called as “the central dogma” in molecular biology. However, in 2000s non-coding RNA was found to be located at the upper regulating stream and control over mRNA expression from DNA was clarified. In addition, the presence of an epigenetic factor in DNA has been proposed. That is to say, microRNA exerts influence over gene expression through DNA-methylation and histone-modification-related intranuclear chromatin-remodeling. Knowledge of genomics theory alone, which only targeted mRNA expression, became unable to explain changes in the phenotype at this level of gene regulation because the process transcends the actions of mRNA. This sort of new molecular toxicological approach toward the genome proposes other gene expressions under a control of non-coding RNA and epigenetic system, and is indispensable for elucidation of the mechanisms of toxic expression.

EXTRAPOLATION TO HUMANS FROM TOXICOLOGICAL DATA AND PROGRESS TOWARD SIGNIFICANT TRANSLATIONAL RESEARCH (FIG. 5, FIG. 6, FIG. 7)

Drug discovery and development from the perspective of translational research

In drug development, the past success rates of drug therapies in most treatment areas (cardiovascular disor-
ders, infection, eye disorder, metabolic abnormalities, pain, etc.) have risen to 11% from 1990 to 2000, and then up to 18% by 2009. However, compared to these diseases for which the outlook for treatment and improvement is sufficiently hopeful, there are still existing as insufficient disease areas for cancer and CNS diseases (the success rate is about 5%). Under this background, it is clear that the challenge in drug development lies in responding to these diseases areas that have yet to be sufficiently dealt with (Kola and Landis, 2004; Walker and Newell, 2009).

As a basic consideration for the creation of new drugs in drug development strategies, the following questions have been raised again: “Is this the correct target for the cause of the disease?” “Has the correct compound been selected?” “Has the correct patient been selected?” On these questions, new scientific and technological approaches for patient-oriented drug discovery and development are addressed to diagnosis-based treatment.

Right target: setting of the correct drug target for the disease

The followings are required in order to set a scientifically innovative target for the cause of the disease: 1) clarification of the target molecule, biological significance and genetic characteristics of the target molecule, 2) presentation of its relationship to known or hypothesized diseases (etiology), 3) identification of its distribution in the target tissue, 4) understanding of the animal species differences in the target, 5) presentation of the clinical target, and 6) technological implementation in the hypothesized test system. Presentation of the mechanism of action compared to known mechanisms and presentation of the possibility of biomarker establishment are also required at selection of a possible target.

Right molecule: selection of appropriate molecule for the target

When selecting a candidate compound for clinical development, the pharmacological and safety profile of the target is clarified, the optimal therapeutic molecule or compound is selected, and the compound with the highest potential for therapeutic effect on the disease is selected. At that time, in order to elucidate the mechanisms involved in drug efficacy and safety, the reaction mechanisms and causal relationships between the target and the relevant molecules are clarified. Based on the results of exploratory safety testing conducted to avoid adverse effects from the point of view of medicinal efficacy and toxicity, accurate risk assessment and risk management of...
Principle of safety evaluation in medicinal drug

Fig. 6. Approaches toward precision medicine based on pathogenesis variation.

Fig. 7. Precision medicine and biomarker plans for intended patients.
the compound are performed.

Right patients: correct selection of patients for treatment subjects

In order to create a clinically effective and safe therapeutic drug, the disease agent target is clarified clinically and in terms of basic biology, and patients who will respond are selected and treated. For pharmaceuticals that are created by making full use of technology and the latest scientific findings (molecular biology and molecular genetic approach) obtained using human samples to profile the relationship between non-clinical and clinical target genes and, it is required that patients with high reactivity be selected and enrolled in clinical trials and treatment.

Investigating and establishment of biomarkers for translational research

When considering the extrapolation of non-clinical study results to humans, the necessity of translational research is discussed in the course from non-clinical to clinical applications by establishing biomarkers. These biomarkers are for medicinal efficacy and safety. As biomarkers for safety assessment, for example, transaminase has conventionally been utilized for liver toxicity and creatinine and urea nitrogen for renal toxicity, but they were insufficient for use in the exploratory research phase in terms of sensitivity and specificity. It is hoped that new biomarker will be established to overcome these limitations. In recent years, the investigation of genomic biomarkers as safety biomarkers has progressed. Viewed from the perspective of the possibility of extrapolation to humans, there are still many problems with the sufficiency of translational biomarker establishment and actual clinical applicability, and those biomarkers with high usability in the clinic, including the post-marketing period, are desired. In addition, in pre-clinical exploratory toxicity studies, not only those related to the toxicity expression of major organs such as the liver and kidney, but also the investigation of precise toxicological biomarkers in other organs is an important issue. In addition, biomarker establishment based on analysis from the perspective of pathway-analysis, adverse outcome pathways (AOP), and systems toxicology— with these areas as part of the overall elucidation of the mechanism of toxic expression for the target (on-target and off-target)—is being aggressively called for (Bureeva and Nikolsky, 2011; Viken, 2013; Bouhifd et al., 2014; Titz et al., 2014; Chappell et al., 2016; Edwards et al., 2016; Furihata et al., 2016). With the progress of gene analysis technology, presentation of biomarkers associated with gene expression has increased since 2000. While some biomarkers were useful in the clinical field, in actual treatment they were not able to present sufficient effects as markers.

Currently, adequate presentation for in vitro diagnostic biomarkers based on molecular biological investigations (proteomic and genomic profiles) has not yet to be achieved because of gene-expression complicity (Poste, 2011). In recent years, contribution to the creation of drugs showing specific treatment effects allowed by the gene diagnosis of causes of disease, biological understanding of diseases based on biomarker discovery, identification of gene mutations as adaptive reactions, and companion diagnostics and the relevant drug development have been proactively incorporated. Understanding disease status and its biological significance and establishment of biomarkers for elucidating the relationship between the cause of disease and its treatment play important roles.

Beginning in the 1970s with the rapid development of the gene recombination technology, in the period where the improvement of gene analysis technology led to the complete analysis of the human genome in 2000. During these decades, improvements were made in the analysis and understanding of disease (causes of disease), and developments began in “Personalized Medicine”, in which the type of disease for each person is observed, and “Precision Medicine”, in which personalized medicine and disease prevention for causes of diseases in individual patients are considered. Against this background, translational research bears a large part of safety assessment of these new medical treatments (Spear et al., 2001; Davies et al., 2002; Kolch, 2005; Rikova et al., 2007; Soda et al., 2007; Barber et al., 2010; Verhaak et al., 2010; Chapman et al., 2011; Pao and Girard, 2011; Horii, 2016).

COMPREHENSIVE OVERVIEW OF SAFETY EVALUATION: SAFETY FROM THE EARLY STAGE OF DRUG DISCOVERY AND DEVELOPMENT TO CLINICAL APPLICATION FOR APPROVAL AND MARKETING

Safety evaluation in each phase of the drug discovery and development: exploratory research for initial drug discovery, selection of candidate compound for clinical development, application for approval, post-marketing

Safety evaluations in the drug development process focus on the toxicity studies hitherto mainly required for IND and NDA approvals. Namely they have been developed for the purpose of assessing the safety of a drug before its first-in-human use for Phase I clinical trials and
for safety assessments and management when the NDA is made, reviewed, and approved from the aspects of regulatory science. On the other hand, investigative exploratory safety studies proceeds from the early discovery phase for lead compound modification and clinical candidate compound selection. Recently, requirements for safety evaluations for these steps have emerged, and the assays are increasingly being arranged as High-Throughput Toxicology (HTP-Tox) screening programs. The current classification of toxicity testing required for each stage of the development process from drug discovery through to clinical development and marketing is as follows: 1) early-phase toxicity testing for drug discovery, 2) exploratory toxicity testing for selection of the candidate compound for clinical trials, 3) safety toxicology studies for first-in-human clinical trials [entry-into human (EIH) testing and IND], 4) toxicity studies for NDAs, and, 5) toxicology testing for post-marketing safety surveillance. The testing and evaluation methods differ at each of these stages, and the corresponding safety endpoints and established biomarkers also differ.

Risk assessment and management at each stage of drug discovery/development: go/no-go decision (Fig. 8)

Risk assessment and management for the relevant compound are carried out based on elucidating the mechanisms of toxicity from multidisciplinary standpoints for the specific toxicities of that compound. Ideally, a compound with a sufficient effect and minimal toxicity should be selected. In drug discovery at the initial stage, toxicological screening is performed, and after lead compound modification the appropriate candidate compound is selected for clinical application. Induced toxicity is investigated by initial estimations from the points of the structure-activity and structure-toxicity relationships based on their toxicological endpoints and biomarkers. A number of factors are involved in the go/no-go decision whether to continue or discontinue development. In many cases, two major factors form the basis for taking this decision: risk-benefit assessment and consideration of unmet medical needs and safety issues to patients. Risks and benefits are assessed in investigations with high level science and technology and evaluations from multiple perspectives from pharmacology, toxicology, pharmacokinetics, drug formulation, and other disciplines. In particular, safety concerns have been the cause of project discontinuation in many cases. Accordingly, detailed safety evaluations are performed at each stage of the process of pre-clinical and clinical study, which naturally covers drug development when it is in progress, but also extends to the post-marketing phase. In addition to this assessment, in the risks and benefits for patients, significant treatment-value with existing therapies and the pharmacovigilance plan are essential for the decision-making process. Insufficiency for understanding of practical extrapolation

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Fig. 8. Safety assessment/evaluation for risk assessment/management.
to humans, safety margin, adverse drug reaction mechanisms, biomarker establishment, and the reversibility of adverse drug reactions has been major decision-points in the cases of project discontinuation mentioned above.

**Drug safety evaluation and guidelines**

The safety evaluations in a drug’s approval process laid out in its application (IND and NDA) documents are based on the results of safety studies (toxicity studies) conducted in accordance with guidelines and regulations stipulated in the Japanese pharmaceutical affairs law. Essentially, the requested submissions of the details on the conduct of the studies and their results as IND and NDA dossiers are timed as shown in the ICH-M3 guidance. The guidelines on submission for regulatory approval represent the base of regulatory science. Regulatory science evaluations enable assessments which are as universal and comprehensive as possible and in which knowledge of the toxicology involved in updated safety assessments. However, at the current moment in time, the data of exploratory research driven by novel sciences are not fully referred, and only used as reference data. Nevertheless, there are many instances where mechanisms of toxicity have been elucidated, biomarkers or endpoints indicative of major adverse reactions have been established, and contributions to risk assessment and management have been made in exploratory research. Conversely, there is a widespread tendency for the category of regulatory science to be limited to mainly describing the adverse reactions and their severity.

The importance of standardization in regulatory science and the need of the challenges of novel scientific developments are generally obscured in the gap between regulatory science and basic science. Basic science is fundamental to regulatory science, and it goes without saying that incorporating new science and technology into regulatory science is essential for its progress into applied science; it is also no exaggeration to say that this progress may depend on whether it constantly continues to adopt the latest scientific developments. We may say that the future position of regulatory science would be bound up in the question of to what extent it requires freeing from an overriding preoccupation with guidelines.

The studies recommended by guidelines for IND and NDA safety evaluations are primarily concerned with the detection of toxicity after exposure to a drug under the conditions of a fixed protocol, and specifying and establishing the severity of this toxicity. In this way, we can see that the ability to detect toxicity may vary depending on the study protocol (which specifies the dose, duration of exposure, parameters to be examined, and other details) and the content of the standard operating procedures (SOP) being applied. In the case of safety evaluations and approval, the no-observed-effect levels (NOAEL) of the relevant studies will inevitably vary due to these factors, and we also need to be aware that the therapeutic index (TI) may vary.

There are following toxicity tests/studies other than the studies conforming to the guidelines covering applications for approval stated above: exploratory toxicity testing for drug discovery, studies for the elucidation of mechanisms of toxicity, and basic scientific research geared to investigating pharmacological and toxicological biomarkers. The objectives of this sort of research could be to elucidate a mechanism of toxicity, make an extrapolation to humans, or establish meaningful biomarkers; therefore, the content of the relevant protocols and SOPs can be determined on a case-by-case basis, and these studies can be conducted incorporating the latest scientific knowledge and techniques. In many cases, basic data are collected in order to select a candidate compound for development based on consideration of the abatement or avoidance of toxicity, or to progress to risk assessment and management considering the elucidated mechanisms of toxicity. Generally, this basic scientific knowledge is frequently applied at the time of go/no-go decisions in drug development, and is treated as reference data in evaluations for regulatory science. In recent years, regulatory authorities of foreign countries are tending to place some weight on such basic scientific data in their reviews of applications for approval as the FDA does.

**Submission of data related to the IND and NDA safety provisions and utilization of their data for reflection in the development safety update report (DSUR), periodic safety update report (PSUR), and risk management plan (RMP) (Fig. 9)**

The process of pharmaceutical discovery and development is scientifically and technically challenging. This process starts with the selection of a “safe therapeutic target” as an effective and very safe candidate compound for clinical development and release onto the market, and it involves the taking of a series of complex decisions through the drug safety assessments for the first-in-human use/Phase I clinical trials to the reviews for approval and actual approval after Phase II and III clinical trials.

The safety assessments (toxicity studies) required at each stage of the process from drug discovery to clinical development and marketing were generally addressed. The safety evaluations for these stages of the process vary somewhat in their content; however, the basic scientific approach to risk assessment and management is the same.
at each stage. The safety assessment items outlined above generally become the central focus of safety evaluations in guideline-compliant studies (GLP studies); however, in many cases the data from exploratory research such as elucidation of mechanisms of toxicity and basic scientific investigations at the initial phase, drug discovery, are also scientifically important. These data can be used in IND and NDA dossiers, but they can also be reflected in DSURs, PSURs, and RMPs, and we can expect that they will also be utilized in post-marketing safety surveillance. An aggregation of data as follows is always required by regulatory authorities at the time of application for approval: a multiplicity of non-clinical assessment data (pharmacological and toxicological assessments: interpretation of data on the respective profiles, mechanism of action, and therapeutic niche; safety assessments: disease model, mechanism of action, pharmacokinetics, comparisons with analogous drugs), extrapolation to humans (relationship between clinical and non-clinical data), risk assessment and management, and suitability of package insert precautions (including information appropriate for specialist physicians).

BASIC ROLE OF SAFETY ASSESSMENT IN DRUG DISCOVERY AND DEVELOPMENT

From a toxicological perspective, the following principal points should be addressed in the safety assessment of pharmaceutical products:

1. In what type of toxicological studies or examinations showed the toxicities?
2. What type of adverse reactions was observed?
3. Were adverse reactions on-target or off-target? (Extended drug efficacy?)
4. How about the strength of the adverse reactions?
5. Was the toxicities reversibility?
6. How about the NOAEL?
7. How about the scientific explanation for extrapolation to humans?
8. How about the risk assessment and management from a comprehensive scientific standpoint?

Based on the above principle consideration of estimated toxicities, the following practical role of toxicology would be listed (Fig. 10). The role of safety assessment in drug discovery and development has always been a matter of “go”, or “no-go”.

- Estimation of discovery target from the aspect of effi-

Fig. 9. Utilization of data in pre-clinical investigative safety study for human prediction.
cacy and safety
- Identification of toxicity (On-target, Off-target)
- Exploratory/investigative toxicological assessment for (1) Investment from the early stage to late stage of drug discovery (2) Elucidation of mechanism of toxicity (3) Avoidance of toxicity based on relationship between chemical structure and toxicity (4) Setting endpoint and related biomarker (5) Selection of lead compounds
- Candidate selection for clinical application
- Implementation for pre-, non-clinical safety study for regulatory submission of IND/NDA
- Risk evaluation/management for post-market

CONCLUSION

The toxic effect of a drug is expressed as a defensive reaction in the body against a foreign substance. The toxic effect caused by a foreign substance can be said to be a trigger to an abnormal condition, or additional or accelerating action leading to such an abnormal condition in contrast with the course of normal function maintenance to avoid abnormal conditions. This can be seen as the logical framework that links evolutional toxicology as a response to evolutional medicine. It is also important to approach an evaluation of a compound from the angle of why exposure to it causes toxic effect expression. Thought of evolutional toxicology helps us to easily understand how drugs work as triggers of toxic effects and how toxic effects are induced, in a drug safety evaluation. It offers the prospect of a valuable consideration of the reaction to toxic effects for which appropriate risk assessment and risk management would otherwise prove complicated. It is thought that, with risk assessment and risk management, go/no-go decision for drug development would be made as to whether the toxic effect of the relevant drug is critical or manageable, and as to how to avoid risks of drug toxicity in an evaluation from the perspective of evolutional toxicology. To have a significant evaluation of toxicities, clarification of on-target or off-target toxic effects is important, and multidisciplinary scientific approaches lead to right decision for safety evaluation in drug discovery and development.

In recent years, scientists have strived to clarify toxic effect expression mechanisms with the introduction of new fields of research such as molecular toxicology. The trigger of the expression of the toxicity lies on gene expression. Through the evaluation of multidisciplinary sciences, the mechanism of toxicity expression would be clarified from the aspect of molecular toxicological assessment using the most recent science and technology. The need to comprehend toxicity for stringent risk assessment and sensible risk management has increased as a systems toxicology based on the analysis of AOP.

As a basic consideration for the creation of new drugs in drug development strategies, strategic concepts to related targets/projects are indispensable, namely the questions are “right target to the cause of the disease?”, “right selection of compound?”, “right selection of patients?” On these questions, new scientific and technological approaches for patient-oriented drug discovery and development are addressed to diagnosis-based treatment such as...
as precision medicine.

Present regulatory science has a trend toward regulation itself, not mainly in science. The safety assessment/evaluation items become the central focus on safety evaluations in guideline-compliant studies; however, in many cases the data from exploratory research such as elucidation of mechanisms of toxicity and basic scientific investigations at the initial phase of drug discovery, are also scientifically important for realistic risk evaluation/management. The data driven from guideline-base are to be mainly used in IND and NDA dossiers, and further they would also be reflected in DSUR, PSUR, and RMP. In order to have a significant risk assessment/management, the science-oriented and patient-oriented assessments are to be desired beyond the present guideline-based regulatory science.

The role of drug safety evaluation as a contribution of toxicology is to be addressed in each stage of drug discovery and development for go or no-go decision. They are estimation of discovery target, identification of toxicity, exploratory/investigative toxicological assessment such as toxicity screening, elucidation of toxicity mechanism, avoidance of toxicity, and setting endpoint and related biomarker, selection of lead compounds, candidate compound selection for clinical application, implementation for pre-, non-clinical safety study for regulatory submission of IND/NDA, and risk evaluation/management for post-market.

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Principle of safety evaluation in medicinal drug

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