**Review**

**Drug-Induced Idiosyncratic Hepatotoxicity: Prevention Strategy Developed after the Troglitazone Case**

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**Summary:** Troglitazone induced an idiosyncratic, hepatocellular injury-type hepatotoxicity in humans. Statistically, double null genotype of glutathione S-transferase isoforms, GSTT1 and GSTM1, was a risk factor, indicating a low activity of the susceptible patients in scavenging chemically reactive metabolites. CYP3A4 and CYP2C8 were involved in the metabolic activation and CYP3A4 was inducible by repeated administrations of troglitazone. The genotype analysis, however, indicated that the metabolic idiosyncrasy resides in the degradation of but not in the production of the toxic metabolites of troglitazone. Antibody against hepatic aldolase B was detected in the case patients, suggesting involvement of immune reaction in the toxic mechanism. Troglitazone induced apoptotic cell death in human hepatocytes at a high concentration, and this property may have served as the immunological danger signal, which is thought to play an important role in activating immune reactions. Hypothesis is proposed in analogy to the virus-induced hepatitis. After the troglitazone-case, pharmaceutical companies implemented screening systems for chemically reactive metabolites at early stage of drug development, taking both the amount of covalent binding to the proteins in vitro and the assumed clinical dose level into consideration. At the post-marketing stage, gene analyses of the case patients, if any, to find pharmacogenetic biomarkers could be a powerful tool for contraindicating to the risky patients.

**Keywords:** troglitazone; chemically reactive metabolite; idiosyncratic drug reactions; drug-induced liver injury; prevention

**Idiosyncratic hepatotoxicity caused by troglitazone**

Troglitazone (Fig. 1 for the chemical structure and metabolic pathway) was introduced into the Japanese and U.S. market in March, 1997 as an innovative new drug for the treatment of type II diabetes mellitus. In December of the same year, however, a doctor letter warning the drug-induced hepatotoxicity by troglitazone was issued. In 2000, troglitazone was withdrawn from the market voluntarily by the pharmaceutical companies in both countries, Sankyo Co., Ltd. and Warner-Lambert Co. Kuramoto et al., investigated 35 Japanese cases of the troglitazone-induced hepatotoxicity including 4 death cases, which were reported to the Ministry of Health and Welfare.\(^1\) The patients were 19 females (age: 46–77 years) and 16 males (age: 34–75 years), and they were taking daily 400 mg-troglitazone (200 mg b.i.d.). The hepatotoxicity was accompanied with increased activities of serum transaminases (glutamic-oxaloacetic transaminase, GOT, and glutamic-pyruvic transaminase, GPT), and the initial sign of high GOT and GPT activities (more than threefold of the control level) was observed about 2 months after starting the troglitazone treatment. The peak in the number of patients showing the abnormality was found in 17 to 20 months after starting the treatment, indicating a slow onset of the hepatotoxicity. Serum level of the total bilirubin also increased markedly, and was regarded as a marker of bad prognosis since the patients with the total bilirubin not more than 5 mg/dL all recovered after discontinuation of the troglitazone treatment while the level of total bilirubin in 4 death cases was more than 8 mg/dL. Increase in the activity of serum alkali phosphatase was not much, and was 1.29-fold the control level. All cases showed no allergic reactions such as fever, rash, and eosinophilia.

The death cases included 3 females (age: 60–66 years) and 1 male (age: 58 years) and had no previous history of hepatitis virus infection. History of drug allergy was found in 2 female death cases, and a positive reaction in the drug lymphocyte stimulation test (DLST, or lymphocyte trans-
formation test: LTT was found in the male death case. Unfortunately, the levels of serum transaminases had not been frequently monitored in these patients as indicated by the severe hepatic dysfunction already evident when checked by the first measurements. The patients showed a hepatic encephalopathy as a consequence of a high total bilirubin level more than 20 mg/dL at the end stage, and died of a fulminant hepatitis.

Preclinical safety assessments using experimental animals conducted in the pharmaceutical company before new drug application failed to predict the troglitazone-induced hepatotoxicity found clinically in humans. Any additional animal experiments conducted extensively after the drug approval were also not predictive of the troglitazone-induced hepatotoxicity in human.2,3

It is accepted that there are 2 types of drug-induced hepatotoxicity in humans, one is the intrinsic hepatotoxicity (Type A hepatotoxicity) and the other is the idiosyncratic hepatotoxicity (Type B hepatotoxicity) and that the former is dose-dependent and reproducible in experimental animals while the latter is dose-independent, and is found only in humans. It should be noted, however, that the idiosyncratic hepatotoxicity being not dose-dependent would be only apparently true in a population containing an extremely small number of susceptible patients in a large number of non-susceptible patients.4

This is because the susceptible patients likely show the toxicity even at pharmacologically effective dose levels, and generally clinical studies are conducted at the therapeutic dose levels; hence, even the lowest dose level is toxic to the susceptible patient. In an assumed clinical trial, where not more than 10000 patients in total are allocated to three therapeutic dose levels, high, medium and low levels, the probability of the susceptible patient, just one patient at the most in this study scale, being allocated to the highest dose group is only 1/3 while the probability of allocation of the susceptible patient to the lowest and medium dose groups is 2/3 leading to a greater chance of the idiosyncratic toxicity manifesting in a dose-independent manner than in a dose-dependent manner. It is thought that, in groups made up only by susceptible patients, toxicity would be detected at a very low dose level, and be dose-dependent as well in a low-dose range.

The idiosyncratic hepatotoxicity is further classified into 2 subtypes, 1) immune idiosyncrasy, where the immune reaction seems to be deeply involved as judged by the fever, rash and eosinophilia accompanied, and 2) metabolic idiosyncracy, where the immune reaction is apparently less important or ignorable.5

Based on these findings and the classification criteria described above, it was concluded that hepatotoxicity caused by troglitazone is an idiosyncratic, hepatocellular injury-type liver toxicity, where the idiosyncrasy is believed to reside in the specific drug metabolism (metabolic idiosyncrasy). The metabolic idiosyncrasy was true at least in part in the troglitazone-case as described later in this review but, at the same time, the involvement of immune reaction was also suggested since the antibody against hepatic aldolase B was detected in the serum samples collected from two patients with troglitazone-hepatotoxicity.6 The positive reaction of DLST found in one death case was also suggestive of the involvement of immune reaction through T lymphocytes.

Fig. 1. Metabolic pathway of troglitazone
In the case of acetaminophen-induced hepatotoxicity, where the immune system is involved in the intrinsic hepatotoxicity as hepatotoxicity seems different. It is likely that the innate immune system plays a role in this type of hepatotoxicity, while the immune system is mainly involved in the idiosyncratic type of hepatotoxicity. The factors separating these two types of drug-induced hepatotoxicity are not known exactly.

Chemically reactive metabolites produced from troglitazone

There are some differences in the causal factors for two types of hepatotoxicity, intrinsic hepatotoxicity and idiosyncratic hepatotoxicity as summarized in Table 1 but, quite interestingly, chemically reactive metabolites are thought to play important roles commonly in both types of drug-induced hepatotoxicity. The factors separating these two types of the drug-induced hepatotoxicity are not known exactly while the immune system mainly involved in each type of hepatotoxicity seems different. It is likely that the innate immune system is involved in the intrinsic hepatotoxicity as in the case of acetaminophen-induced hepatotoxicity, where inflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) predominate over the anti-inflammatory cytokines such as interleukin 4 (IL-4) and IL-10. The adaptive immune system is involved in the idiosyncratic hepatotoxicity as in the case of halothane-induced hepatotoxicity, where sensitization is needed, and many autoimmune antibodies are produced. It could be assumed that the reactive metabolites chemically modify the hepatocellular proteins in varying manners in terms of either different protein species modified, different sites of modification in the same protein or the different rate and extent of covalent binding, leading to a stimulation of different immune system and finally to manifestation of different type of hepatotoxicity, though the precise mechanism is still unclear.

The major metabolites of troglitazone detected in both the experimental animals and humans were glucuronide and the sulfo-conjugate of troglitazone (Fig. 1), which were obviously not chemically reactive and not assumed to cause the hepatotoxicity. After removal of troglitazone from the market, many studies have been carried out in order to explore the chemically reactive metabolites of troglitazone in vitro, and several reports described the production of chemically reactive metabolites from this drug. As shown in Figure 2, these are the troglitazone semiquinone radical, quinone methide-form metabolite, sulfenic acid-form metabolite, α-ketoisocynate-form metabolite, epoxide-form metabolite, and thiazolidine dione radical (Fig. 2). The troglitazone semiquinone radical is not demonstrated directly for troglitazone but is thought quite likely produced in analogy to other quinone-form compounds. In addition to these observations, 14C-labelled troglitazone was found to bind covalently to the proteins in primary cultured human hepatocytes. All these findings clearly demonstrate that the chemically reactive metabolites are produced from troglitazone in the liver in addition to the production of the stable, major metabolites. CYP2C8 and CYP3A4 are likely the most important isozymes of cytochrome P450 in bioactivating troglitazone. However, the involvement of CYP3A4, the most major isozyme of cytochrome P450 present in human liver, in producing the chemically reactive metabolites is more or less puzzling when considering a low incidence rate of the troglitazone-induced idiosyncratic hepatotoxicity, which is about one among more than 30000 patients. Although CYP3A4 shows great inter-individual variability and high CYP3A4 activity would produce an increased amount of chemically reactive metabolites and hence the increased risk of hepatotoxicity in some individuals, it goes reversely with an extremely low incidence rate of idiosyncratic hepatotoxicity caused by troglitazone. Several papers demonstrate that troglitazone is a potent inducer of CYP3A4 in human liver both in vitro and in vivo. The idea of metabolic idiosyncrasy suggests that a limited number of patients have a specific metabolic pathway leading to a rare toxicity while CYP3A4 being the most major cytochrome P450 isozyme in human liver and its activity being inducible by repeated administration of troglitazone would make troglitazone to be

Table 1. Comparison of intrinsic hepatotoxicity and idiosyncratic hepatotoxicity

<table>
<thead>
<tr>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic hepatotoxicity</td>
<td>Idiosyncratic hepatotoxicity</td>
</tr>
<tr>
<td>Genetic factor</td>
<td>-</td>
</tr>
<tr>
<td>Dose-dependency</td>
<td>+</td>
</tr>
<tr>
<td>Reproducibility in animals</td>
<td>+</td>
</tr>
<tr>
<td>Onset</td>
<td>Rapid (level of days)</td>
</tr>
<tr>
<td>Chemically reactive metabolite</td>
<td>+</td>
</tr>
<tr>
<td>Major immune system involved</td>
<td>Innate immune system</td>
</tr>
</tbody>
</table>

*Genes involved have not been identified in most cases. Multiple genes are believed to be involved.

1Idiosyncratic hepatotoxicity would be dose-dependent in the susceptible patients.

2The innate immune system is also likely involved in idiosyncratic hepatotoxicity.

Fig. 2. Chemically reactive metabolites produced in vitro from troglitazone

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hepatotoxic in a greater number of patients rather than in a limited number of patients. Therefore, genetic variability in the oxidative metabolism producing the chemically reactive metabolites would not be a causal factor for the idiosyncratic nature of troglitazone-induced hepatotoxicity.

Gene analysis of the case patients: Decreased activity of glutathione conjugation as a risk factor

Watanabe et al. reported the results of a genetic polymorphic analysis using blood samples collected from 25 Japanese patients with type II diabetes mellitus who experienced the troglitazone-induced hepatotoxicity showing more than fivefold increase of GOT and GPT (the case patients) and 85 diabetic Japanese patients who were taking troglitazone without any sign of hepatotoxicity (the control patients).21) Genotype analysis was performed at 68 polymorphic sites of 51 candidate genes related to drug metabolism, apoptosis, production and elimination of reactive oxygen species, and signal transduction pathways involved in the oxidative metabolism producing the chemically reactive metabolites, and limited number of patients. Therefore, genetic variability in the oxidative metabolism is not a risk factor statistically for drug-induced hepatotoxicity. In fact, as shown in Table 2, it should be noted that the double null genotype of GST is found in 13 troglitazone-tolerant, control patients (15%) and the double wild genotype of GST is found in 3 case patients (3%), clearly indicating that the GST double null genotype solely is not an overriding determinant of the troglitazone-induced hepatotoxicity and that polymorphism of unknown other genes is also involved.

Genetic polymorphisms reported in relation to idiosyncratic drug reactions: Importance of polymorphism of human leukocyte antigen (HLA) genes

Although the GST double null genotype is no doubt the risk factor statistically for drug-induced hepatotoxicity, the risk of the GST double null genotype carrier is only about threefold compared with the non-carrier, and does not elucidate a low incidence rate of the troglitazone-induced idiosyncratic hepatotoxicity. In fact, as shown in Table 2, it should be noted that the double null genotype of GST is found in 13 troglitazone-tolerant, control patients (15%) and the double wild genotype of GST is found in 3 case patients (3%), clearly indicating that the GST double null genotype solely is not an overriding determinant of the troglitazone-induced hepatotoxicity and that polymorphism of unknown other genes is also involved.

**Table 2. Genotype analysis of glutathione S-transferase (GST)**

<table>
<thead>
<tr>
<th>Genotype of GST</th>
<th>Control (tolerant patients)</th>
<th>Case patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTT1 GSTM1</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Wild type Wild type</td>
<td>25 (29)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Wild type Null</td>
<td>27 (32)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>Null Wild type</td>
<td>20 (24)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Null Null</td>
<td>13 (15)</td>
<td>10* (40)</td>
</tr>
<tr>
<td>Total</td>
<td>85 (100)</td>
<td>25 (100)</td>
</tr>
</tbody>
</table>

*P < 0.01

The Stevens-Johnson syndrome is known as one of the severe, idiosyncratic adverse skin reactions caused by drugs. A very strong correlation of the polymorphism in the major histocompatibility complex class I gene (MHC-I or human leukocyte antigen, HLA) with the carbamazepine-induced Stevens-Johnson syndrome has been reported for the Han Chinese.24) The genotype, HLA-B*1502, was found in 100% of patients susceptible to the carbamazepine-induced Stevens-Johnson syndrome while it was found only in 3% of the carbamazepine-tolerant patients (odds ratio: 2504). The same research group also reported a strong correlation of HLA-B*5801 with allopurinol-induced skin toxicity for the Han Chinese (odd ratio: 580).25) With regard to the drug-induced hepatotoxicity, a strong correlation of HLA-A*3303 with idiosyncratic, cholestatic hepatotoxicity induced by ticlopidine in the Japanese patients (odds ratio: 36.5) and of HLA-B*5701 with idiosyncratic hepatotoxicity induced by fluoroacillin in the European patients (odds ratio: 80.6) has been reported.26,27) The odds ratios are quite high in these studies, and, therefore, by contraindicating the carriers of known HLA-genotypes correlated with the specific drug-toxicity, it is possible to prevent clinically the occurrence of idiosyncratic drug toxicity. In fact, FDA is recommended to screen the patients for HLA-B*1502 before starting the treatment with carbamazepine.28 This strategy was successfully applied to avoid prospectively the abacavir-induced skin hypersensitivity, and the treatment of only the HLA-B*5701-negative patients resulted in no occurrence of the hypersensitivity reaction.29)

It should be noted, however, that HLA genotype involved in the drug toxicity seems ethnicity-specific, and, as an example, HLA-B*1502 is not predictive of the carbamazepine-induced Stevens-Johnson syndrome in the Caucasian and Japanese patients.30,31) It should be also noted that the HLA genotype related to the drug-induced adverse reactions differs drug by drug, and must be determined in a case-by-case manner. By contraindicating the high risk patients...
according to the HLA genotype determined for each drug and for each ethnicity, therefore, drug-induced idiosyncratic adverse reactions could be prevented. Not only HLA genotypes serve as biomarkers of the drug-induced toxicity but also other genotypes, such as those of drug-metabolizing enzymes and transporters, do so, and the use of more than one pharmacogenetic biomarker, when available, would be effective in preventing idiosyncratic drug-toxicity. \(^{32}\)

**Hypothesized mechanism:**

**Analogy of drug-induced hepatotoxicity to virus-induced hepatitis**

The fact that MHC-I gene polymorphism is highly related to the idiosyncratic drug-induced adverse reactions including hepatotoxicity strongly suggests that the immune system is somehow working in these reactions. MHC-I is involved in self and non-self recognition as indicated by the fact that, in the organ transplantation, HLA type-matching is crucially important to cut down the risk of rejection, which is the immune-mediated reaction to eliminate non-self from self through the action of cytotoxic T lymphocytes. However, it is still not known precisely how the immune reaction is involved through MHC-I proteins in idiosyncratic drug-induced hepatotoxicity, although it is believed that hepatic proteins chemically modified by the reactive metabolites would trigger a series of immune reactions.

As a hypothesis, the mechanism for viral hepatitis could be applied to idiosyncratic, drug-induced hepatotoxicity. \(^{33,34}\) Many hepatitis virus carriers are free from the symptom, and a portion of the carriers develops the hepatitis much delayed after viral infection, exhibiting a similar feature to idiosyncratic drug-induced hepatotoxicity. In the viral hepatitis, it is thought that peptide fragments are produced from the viral proteins by a proteasomal processing, taken up by endoplasmic reticulum of hepatocytes by the action of transporter associated with antigen processing (TAP), bound within a groove of MHC class I protein in the endoplasmic reticulum, and the virus peptide - MHC class I protein complex is transported to the cell surface through the Golgi apparatus, exhibiting this foreign peptide to the immune system. Cytotoxic T lymphocytes recognize the virus-derived foreign peptide caught on the groove of MHC class I protein and kill the hepatocytes, regarding these cells to be foreign as a whole. \(\text{Figure 3}\) shows schematically the hypothesized mechanism for the drug-induced hepatotoxicity constructed in analogy to the viral hepatitis. The drug is oxidized by cytochrome P450 (CYP) to the chemically reactive metabolite (pathway 1), which is ordinarily detoxified by the action of glutathione S-transferase (GST). Under the condition of induced cytochrome P450, null GST genotype or glutathione depletion, the chemically reactive metabolites increase, and bind covalently to hepatocellular proteins (pathway 2). When covalent binding occurs on important proteins for cell function, the hepatocytes will be killed either by severe cell dysfunction as a direct action or by the activation of innate immune reaction leading to the predominance of the inflammatory cytokines over the anti-inflammatory cytokines (pathway 3). The dying cells or activated immune system could emit the immunological danger signal as described later. Chemically modified proteins undergo break-down by proteasomal processing to peptide fragments, one of which being modified by the reactive metabolite (pathway 4). The peptide fragments are transported by TAP into the endoplasmic reticulum (pathway 5), and each peptide binds to MHC-I protein specific to each fragment (pathway 6). Most probably, the peptide fragment modified by chemically reactive metabolite may not have the counterpart MHC-I protein but the MHC-I protein with a genetic variation may bind to the modified peptide (pathway 6). MHC-I–peptide complexes are moved to Golgi apparatus, and subsequently to the cell surface of the hepatocytes (pathway 7 and pathway 8). The peptides with normal amino acid sequences would be immunologically ignored due to their “self” nature. On the other hand, the “non-self” peptide fragment modified by the chemically reactive metabolite is foreign to the immune system, and after receiving somehow the danger signal (pathway 9), cytotoxic T lymphocytes (CTL in \(\text{Fig. 3}\)) will increase in number and start binding to the hepatocytes through interaction between MHC-I protein and T cell receptor (pathway 10). Cytotoxic T lymphocytes kill the hepatocytes by 3 ways (pathway 11) all causing apoptotic cell death (pathway 12), 1) by expressing Fas ligand, which binds to Fas on the hepatocyte surface, 2) by expressing TNF-α, which binds to TNF-α receptor and 3) by releasing perforin, which polymerizes on the hepatocyte surface to form a channel, and an apoptotic serine-protease, glanzyme, which enters the hepatocyte through the channel.

It is known that the immunological danger signal is needed to activate the immune reactions (pathway 9), and is thought to play an important role in the idiosyncratic drug toxicity. \(^{4,35,36}\) It is not exactly known what the danger signal is but the dying cells are said to emit the danger signal. \(^{37}\) With regard to this point, troglitazone causes apoptotic cell death in rat and human hepatocytes and other cells at a high concentration more than 50 µM, \(^{38-41}\) and this may have served as the danger signal in the mechanism of troglitazone-induced hepatotoxicity. The major metabolite, troglitazone sulfate has been reported to inhibit competitively the bile acid exporting pump (Bsep) with a low Ki value of 0.23 µM, possibly causing a hepatocellular accumulation of bile acids, which are cytotoxic endogenous substances to hepatocytes. \(^{42}\) This also may have assisted the apoptosis, and served as the danger signal.

The number of the circulating cytotoxic T lymphocytes, that recognize the drug-modified peptide as foreign substance, would be small, and the danger signal likely stimulates the proliferation of these cells. Cytotoxic T lymphocytes increased in number are thought to cause many
cell deaths propagated to a whole liver and consequent severe hepatotoxicity. This process is accompanied with cell proliferation, and needs time and hence the delayed toxicity. None of these processes in this hypothetic mechanism in Figure 3 has been demonstrated experimentally for the drug-induced hepatotoxicity but the pathways 1, 2 and 3 to pathway 12 may constitute the intrinsic hepatotoxicity and the pathways 1, 2, 4, 5, 6, 7, 8, 9, 10, and 11 to pathway 12 may constitute the idiosyncratic hepatotoxicity. We know that the mechanism for the drug-induced idiosyncratic hepatotoxicity is quite complicated, and the above described hypothesis would cover only a portion of the mechanisms for drug-induced idiosyncratic hepatotoxicity and future modification of or addition to this hypothesis should be awaited.

**Drug development and strategy for preventing idiosyncratic drug-induced hepatotoxicity**

Unlike the intrinsic hepatotoxicity (type A hepatotoxicity), idiosyncratic drug-induced hepatotoxicity could occur repeatedly in future since preclinical animal experiments are not predictive of such toxicity. Drug withdrawal caused by the drug toxicity greatly discredits the pharmaceutical company leading subsequently to many litigations and financial damage.

In a therapeutic area where no effective drug is available, the first-in-class drug could be used clinically even if the drug shows idiosyncratic drug toxicity since it is better to treat a majority of no risk patients who need the treatment rather than to apprehend the drug toxicity occurring only in quite a limited number of patients. However, the first-in-class drug showing the idiosyncratic toxicity will be replaced immediately by a second generation drug free from such toxicity, which usually comes into the market within a few years following the launch of the first drug. In other therapeutic areas where existing drugs are available, the drug accompanied with idiosyncratic toxicity loses the competitiveness in the market, and the development of such drug does not provide the company with a profit. Therefore, the development of safer drugs is critically important for pharmaceutical companies.

The troglitazone case occurred in 1997–2000, and at that time in late 1990s, screening systems, either conventional or high-throughput, for improving the pharmacokinetic properties of lead compounds began to be implemented in pharmaceutical companies. Since the idea of chemically reactive metabolites as a risk factor of idiosyncratic drug toxicity was disseminated to the research and development people in the pharmaceutical companies, several screening methods for chemically reactive metabolites have been...
introduced. The most straightforward method was to use $^{14}$C- or $^3$H-labeled compound and measure the amount of covalent binding to the proteins by radioassay after incubation with human liver microsomes in the presence of NADPH or with human hepatocytes. This method needs a radiolabeled substance, and could not be employed at an extremely early stage. However, quantitative measurement of the drug-related substances covalently bound to the microsomal protein is rapid and easy by this method, and a well considered criterion in vitro is available (not more than 50 pmole/mg-protein). In addition to the criterion in vitro, Uetrecht reported that a dose level of not more than 10 mg/body/day would not cause drug-induced toxicity, and two judging criteria: 50 pmole/mg-protein in vitro and 10 mg/body/day in vivo could be used conveniently for decision making, although some modification of these criteria has been made as described later.

Measurement of the activities of cytochrome P450 is carried out by a high-throughput screening method using a 96-well titer plate, fluorescent substrate specific to each isoform of cytochrome P450 and robotics device. When a chemically reactive metabolite is produced from the drug substrate, the reactive metabolite inactivates the enzyme, and the inhibitory effect increases as the time of incubation is prolonged. This phenomenon is known as mechanism-based inhibition or time-dependent inhibition, and is utilized in the high-throughput screening to check the production of chemically reactive metabolites.

Other screening methods for chemically reactive metabolites employ trapping agents that react rapidly with chemically reactive metabolites forming stable adducts. Reduced form of glutathione, either unlabeled, stable isotope-labeled, radioisotope-labeled or fluorescence-labeled, is used most frequently as a trapping agent but N-acetyl cysteine, N-acetyl cysteyln lysine, potassium cyanide, semicarbazide and synthetic peptide containing cysteine, lysine and histidine are also used. The adduct formation is detected by radioactivity detection combined with chromatography when the radiolabeled trapping agents are used, by fluorescence detection when the fluorescence labeled trapping agents are used or by liquid chromatography/mass spectrometry or liquid chromatography/tandem mass spectrometry when non-labeled or stable isotope-labeled trapping agents are used.

Information on risky compounds producing chemically reactive metabolites at a level more than the criteria established in each company is to be provided to the chemists for further structural modification to reduce risk. As the situation demands, it would be necessary to isolate and structurally determine the adduct in order to locate the site of adduct formation on the molecule and to find potentially risky chemical structure. This information is quite useful for the chemists to plan a synthetic strategy.

At the early stage of drug development, a “Go/No-Go” decision for each research project would be made generally based on the pharmacological potency of lead compounds or based on the early toxicological and pharmacokinetic data but not based on a future risk of idiosyncratic hepatotoxicity or adverse reactions. However, we need the criteria for production of the chemically reactive metabolites at this stage, considering possible chance of a risky project still being forwarded to the next stage and the compounds with a high risk of idiosyncratic toxicity being placed for further development. As described before, the amount of covalent binding not more than 50 pmole/mg-protein has been advocated as the criterion in vitro. The validity and allowance of this criterion are still not known in avoiding the idiosyncratic drug toxicity clinically, and chemists would be hesitant to strictly comply with this criterion since doing so may eliminate otherwise promising compounds from further development. Nevertheless, it has been reported that known hepatotoxic drugs tend to show a high covalent binding to the protein compared to safer drugs with some exceptions. Therefore, more improved quantitative criterion is needed. Use of only the criterion in vitro for covalent binding is clearly incomplete for making appropriate judgment of idiosyncratic drug toxicity in vivo and the assumed dose level in humans should be also taken together into consideration.

As a more quantitative method for decision making, Nakayama et al. reported a zone classification system based on data on covalent binding collected using the radiolabeled drugs together with the data on daily dose level. As shown in Figure 4, when the log-normalized covalent binding in human hepatocytes is plotted against the log-normalized daily dose, the drugs withdrawn from the market or the drugs with the label of black box warning are mostly located in the unacceptable zone, the drugs with the label of warning are mostly located in the problematic zone, and the safe drugs were located in the

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**Fig. 4. Zone classification for risk assessment of idiosyncratic drug toxicity**

Figure from Nakayama et al. with modification. ( ) Safe drug without any warning (12 compounds), ( ) Drug with a label of warning (14 compounds), ( ) Drug withdrawn or with a label of black box warning (10 compounds).
acceptable zone. Of 36 drugs tested, 30 drugs were located to their respective safety categories (acceptable zone: safe, problematic zone: warning, and unacceptable zone: black box warning or withdrawal), and this zone classification method would be helpful in decision making at the early stage of development.

As another method using the covalent binding and daily dose together, a comparator database on the covalent binding in vitro (B), clinical dose level (D), and absorption ratio (A) would be useful, although such a database has not been constructed. The product of 3 items (B·D·A) serves as a delegate of bodily exposure to the chemically reactive metabolites, and by comparing this value of the compound in question with those values of the drugs withdrawn from the market, the drugs with warning and the safe drugs, a relative position in safety of the compound could be known. Results could be presented that the compound A is much safer than troglitazone and in between amlodipine and rosiglitazone, hence safe, while compound B is risky in between flutamide and diclofenac. Data for the database should be collected according to standardized protocol using a large pool of human liver microsomes in order to obtain quality controlled data over a long period. Since the activity of drug-metabolizing enzymes varies from one lot of human liver microsomes to the other, use of the same lot of human liver microsomes is mandatory.

Strategy for preventing drug-induced idiosyncratic hepatotoxicity would be as follows.

Step 1) the lead compounds for optimization: At early stage of drug development, conduct a high-throughput screening for chemically reactive metabolites. Feed back the data to the chemists for chemical modification of a group of lead compounds to reduce the risk. Repeat this cycle until risky compounds are eliminated from the project, and go to Step 2. When the elimination of risky compounds from the project is unsuccessful, go to Step 3.

Step 2) the case of likely safe compounds: At the stage of drug candidate profiling, determine quantitatively the covalent binding using \(^{14}\)C-labeled compounds. Estimate the clinical dose level by an extensive in vitro-in vivo extrapolation and pharmacokinetic/pharmacodynamic correlation analyses. Confirm the safety of the drug candidate using the potency of covalent binding, estimated clinical dose level and zone classification system.

Step 3) the case of risky compounds: Determine whether a period of treatment by the compounds in question is to be short or long clinically. When the treatment period is short, go to Step 3-1. When the treatment period is long, the compounds are accompanied with increased risk of idiosyncratic hepatotoxicity, and determine whether or not the project is going to develop the first-in-class drug. Go to Step 3-2 in the case of not being the first-in-class drug and to Step 4 in the case of the first-in-class drug.

Step 3-1) the case of risky but acceptable compounds: Very short treatment period in the clinical situation would decrease greatly the risk since more than 1–2 months are normally needed to develop idiosyncratic hepatotoxicity. Therefore, the signal for the project developing drugs to be used only for a short period will be “Go”, disregarding the drug being or not being the first-in-class drug. Even in the case of short clinical use, however, it should be kept in mind that a high dose level may cause other types of idiosyncratic toxicity such as penicillin-induced anaphylactic reactions, which are believed to be due to previous sensitization. Therefore, the zone classification system should be applied to the compound to determine relative safety. Depending on the safety positioning, clinical study to discover the pharmacogenetic biomarkers is better to be planned in future to establish the contraindication to a high-risk patient.

Step 3-2) the case of risky but unacceptable compounds: If the compound to be developed is not the first-in-class drug, and the treatment period is to be long, evaluation of safety by the zone classification method should be carried out as early as possible using \(^{14}\)C-labeled compound. When the safety of the compound is judged problematic or unacceptable, the signal for the project will be “No-Go” because the compound has no competitiveness against the existing low-risk drugs available in the market.

Step 4) the case of risky but clinically useful compounds: On the premise that the compound to be developed is the first-in-class drug having an extremely high efficacy, and no other existing drug is available in the targeted therapeutic area, the signal for the project would be “Go”. At the final stage of lead optimization or drug candidate profiling, determine quantitatively the covalent binding using \(^{14}\)C-labeled compound, and evaluate the relative position in safety of the compound according to the zone classification system as described above in Step 2. When judged risky, it is indeed recommended to carry out a clinical study on pharmacogenetic biomarkers in future to establish the contraindication of susceptible patients. Use of the biomarkers will prolong the life cycle of the drug after being launched into the market.

Conclusion

After the case of troglitazone-induced idiosyncratic hepatotoxicity, various screening methods for the chemically reactive metabolites at the early stage of drug development are implemented in many pharmaceutical companies. Drug candidate compounds that produce no chemically reactive metabolite certainly would have very low possibility of inducing idiosyncratic drug reactions. How effective these screening methods at early stage in preventing the idiosyncratic hepatotoxicity when the drug is given to a vast number of patients is, however, still unknown and needs more time for validation. At the stages of post-marketing surveillance, gene analyses of the susceptible patients, if any, would be helpful to find out the pharmacogenetic biomarkers, and contraindication of susceptible patients would prolong the life cycle of the drug in question.
References


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