

Disease-based Toxicology on Safety Assessment Strategy and Application for Herbal and Traditional Medicines

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Abstract

The safety issue on herbal and traditional medicines (H&TM) is one of the most challenging problems and serious concern worldwide. With scientific endeavor and further exploration, we came to realize that there are great differences between H&TM and synthetic drugs in many aspects, such as medical theory, medication experience, compatibility, processing, toxicological characteristics, and safety evaluating requirements. In the current preclinical models for synthetic drugs, the safety assessment results of some conventional deemed “nontoxic” H&TM were not well consistent with clinical situations, which resulted in major difficulties to understand the mechanisms and guide the safe and rational uses of these H&TM. Thus, based on the traditional Chinese medicine toxicity theory called You Gu Wu Yun, this paper introduces a novel safety assessment strategy for H&TM, named as disease-based toxicology. It aims to cognize the relativity and susceptibility of the toxicity of H&TM, and then to enhance controllability in new drug development and clinical applications. It also provides a theoretical practice for the traditional Chinese medicine toxicity theory and a methodological promotion for the future development of the precision toxicology for H&TM.

Keywords: Disease-based toxicology, idiosyncratic toxicity, safety of herbal and traditional medicines, susceptibility, syndrome differentiation-based toxicity attenuation

INTRODUCTION

With the wide application of herbal and traditional medicines (H&TM) and the improvement of monitoring systems, there is an increasing trend of disclosed adverse drug events or reactions (ADE/ADR) related to conventional deemed “nontoxic” H&TM. Some hot events, such as the Xiao Chai Hu Tang, the berberine, and the aristolochic acid, have raised serious concern all over the world. However, with the limitation of the current theories and strategies, as well as the complexity of H&TM itself, it seems difficult to meet the growing demand and expectation for public safe medication, which is the principle problem hindering development in the field.

With the implementation of the H&TM modernization, there are some crucial progresses on the safety research of H&TM. The evaluation system for preclinical drug safety in line with international norms has been established, and the safety assessment of common toxic H&TM has been carried out, which provide the strong scientific and technological support for the safe use of H&TM. Deserved to be mentioned, focusing on drug-induced

liver injury (DILI), Chinese scholars have established the clinic-associated safety assessment strategy with the evidence chain-based method.^[1] Further improvements were accomplished that the China Association of Chinese Medicine approved the Guideline for the Diagnosis and Treatment of Herb-induced Liver Injury^[2] and the Guidance for Clinical Evaluation of Herb-induced Liver Injury^[3] was enacted by SFDA. However, significant

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differences cause more attention between H&TM and synthetic drugs in medical theory, medication experience, compatibility, processing, toxicological characteristics, and safety evaluating requirements.^[4] Particularly conventional deemed “nontoxic” H&TM, the experimental evaluation results are not consistent with clinical safety, when if the individual status, such as different diseases, syndromes, habitus, genetic factors, and immunological reactions, have not been involved in the safety evaluation system.

Thus, shifting from the conventional thinking pattern, we systematically analyze the differences and similarities in toxic property, medication rules, and evaluating requirements between H&TM and synthetic drugs, and present a novel and disease-associated safety assessment strategy for H&TM, named as disease-based toxicology (DbTox).^[5] It is of great significance to understand the relativity and susceptibility of the toxicity and to enhance controllability in new drug development and clinical applications.

CHALLENGES AND SOLUTIONS FOR SAFETY EVALUATION OF HERBAL AND TRADITIONAL MEDICINE

With decades of development, the preclinical safety evaluation system, which plays an important role in avoiding or reducing severe ADR, has reached international agreements. In fact, drug toxicity is commonly divided into intrinsic toxicity and idiosyncratic toxicity. The intrinsic toxicity can be generally uncovered by conventional toxicological experiments in the preclinical evaluation stage, while the idiosyncratic toxicity is usually observed until the clinical evaluation stage, which could cause severe ADR and drug withdrawal.^[6] With the insidious and sporadic characteristics, inconsistent with the dosage or course of treatment, idiosyncratic toxicity is difficult to predict by conventional evaluation methods. As a result, it becomes a challenging problem and serious concern to assess and predict the idiosyncratic toxicity, which urgently needs a sound evaluation strategy.

Since H&TM have been applied for thousands of years with abundant experience of clinical efficacy and safety, idiosyncratic toxicity is regarded as an important reason for the ADR of conventional deemed “nontoxic” medicines and is closely in correlation with multiple individual’s status (e.g., different diseases, syndromes, and habitus). However, those individual status are difficult to assess by conventional toxicological experiments, where normal animals are the main experimental subject, leading to insufficient translation of the experimental results into clinical application, as well as scant guidance to precision medicine. Therefore, novel strategy and methodology on the safety evaluation for conventional deemed “nontoxic” H&TM are urgently needed.

REVELATION OF THE HERBAL AND TRADITIONAL MEDICINE SAFETY EVALUATION BASED ON THE YOU GU WU YUN

In fact, the Theory of Chinese Medicine enormously emphasizes the syndrome differentiation-based medication, i.e., the individual

state has an impact on the efficacy and safety of medicines. It is also the basic principle to guide the clinical application of H&TM. The You Gu Wu Yun was early proposed as a kind of dialectical understanding of thought in the Plain Questions, with the explanation of that “If you have any disease, your sick body will absorb it as the medicine, otherwise your healthy body will absorb it as the poison.”^[7] It reminds us to consider the individual status and disease when assessing the safety of H&TM. When H&TM works on healthy bodies rather than suitable patients with specific syndromes, even if conventional deemed “nontoxic” medicines, it will be more possibilities to induce injuries. By contrast, if the treatment with the toxic H&TM is the “like-cures-like,” it can have good therapeutic effects with the balance between therapeutic benefits and risks. For example, arsenous acid, which is found from arsenic, has been internationally recognized for its significant effect on acute promyelocytic leukemia.^[8] Nevertheless, if the arsenic preparation is used on healthy people or patients with other indications, it is likely to induce serious toxicity. Another example is that it is appropriate to use a larger dose of *Aconitum carmichaeli* Debx. (fuzi) to “restore the positive and rescue the negative” for patients with the yangjue syndrome, but under other body conditions, the same dose of fuzi can lead to severe toxicity and even to death.

The Wuchangzheng on Plain Questions says that there are patients who can resist the property of some medicines and those who cannot, so physicians should give larger dosage to the former and less to the latter. These words tell us that toxicity responses or side effects could have some significant differences in different individual status. Therefore, we should pay close attention to both the chemical components of H&TM and the effects of different individual status when assessing the safety of H&TM. In addition, the individual’s status includes normal, disease, physical tolerance, susceptibility, and so on. The status of physical tolerance or susceptibility mainly refers to that people with different age, gender, race, and genetic background have differences in metabolism enzyme system and immune-related genetic polymorphism, and then, they present different sensitivity and tolerance to the toxicity of drugs. The individual status has great impact on the safety of H&TM, which has been confirmed by many studies, such as that aconite has less cardiac toxicity on Kidney-Yang insufficiency rats than on normal models,^[9,10] and that *Tripterygium wilfordii* ployglycosidium has more effects on the liver biochemical indicators of normal rats than that of adjuvant-induced arthritic rats.^[11] Thus, it can be seen that the safety assessment of H&TM should attach great importance to the influence of individual status on the toxicity, to more scientifically and objectively evaluate the safe use of H&TM.

HERBAL AND TRADITIONAL MEDICINE SAFETY ASSESSMENT STRATEGY BASED ON THE DISEASE-BASED TOXICOLOGY

Although the safety evaluation system has formed fixed norms, it is mainly aimed to assess the intrinsic toxicity. The evaluation and prediction of idiosyncratic toxicity of H&TM remain blank.

For example, as the idiosyncratic DILI, a kind of ADR within the clinical dose range, it is unrelated to the pharmacological effect of drugs with no obvious dose-response relationship. It only occurs in a small portion of patients, with the incidence between 0.01% and 5%.^[12] There are two mechanisms of idiosyncratic toxicity induced by H&TM, that is, the immune-mediated idiosyncrasy and the metabolic-immune-mediated idiosyncrasy. The former is mainly related to the dysimmunity or immune dysfunction (such as allergies and immunological stress), and the latter has a close correlation with the immune system and genetic polymorphisms of drug metabolic enzymes. According to wider acceptance hapten hypothesis, a drug-active metabolite (hapten) will be covalent binding with the endogenous protein to produce an immunogenic drug-protein complex, which will induce the activation of immune cells and then excessive inflammatory responses and acute injuries. Among them, genetic polymorphism of the immune system is the most frequently revealed mechanism of idiosyncratic hepatotoxicity, and the main target is gene mutation of human leukocyte antigen.^[13] In addition, it is easier to carry out the safety evaluation and risk control to toxic H&TM than conventional deemed “nontoxic” H&TM, because the clinical administration for toxic H&TM is rigorous. While ADRs of conventional deemed “nontoxic” H&TM are insidious and unpredictable, so more research should be explored by systematic and scientific methods.

Based on the toxicity theory of You Gu Wu Yun, a strategy of the DbTox is introduced,^[5] which can evaluate and predict clinical application effects accurately by the animal models with a specific syndrome or disease.^[14,15] Taking the real world and imitative clinical models as assessment tools, the DbTox compares the sensitivity and tolerance to a drug on the different individual status by the assessment methods on systematic toxicology and predictive toxicology. This strategy includes two parts. One is mainly applied for toxic H&TM or H&TM with fierce properties, which is to clarify “syndrome (disease)-dosage-toxicity-effect” relationship of drugs by parallel comparing normal and disease models and to explore the right indications and the possible therapeutic window. The syndrome “(disease)-dosage-toxicity-effect” relationship indicates that the therapeutic effect and side effect of H&TM may be different under different body status and they are intimately associated with usage and treatment cycle. It aims to provide the reference for syndrome differentiation-based precision medication. The other is suitable for conventional deemed “nontoxic” H&TM, which is to evaluate the safety risk of medication to idiosyncratic patients and to reveal the susceptibility factors, related mechanisms, and biomarkers based on susceptible and idiosyncrasy models. It can guide the screening for susceptible population and precision medication.

The syndrome (disease)-dosage-toxicity-effect relationship and therapeutic window directed by the You Gu Wu Yun

Like the old saying, drugs can cure diseases by taking advantages of itself property, sometimes the toxicity. It

means that the toxicity of drugs is conditional. H and TMs can cure diseases under the appropriate application (with right syndrome and appropriate dose), while cause toxicity or side effects if otherwise. Thus, the toxicity of H&TM should not be assessed by a one-sided approach. For example, rhubarb is widely used in the treatment of gastrointestinal disease, liver and kidney disease. However, it has been abroad reported that liver and kidney injuries and potential cancer risks could be induced by taking it for long course with its component of anthraquinones.^[16] The safety of rhubarb and its Chinese patent medicine are questioned. It seems to be a paradox that rhubarb treats chronic hepatitis and nephritis while it could induce liver and kidney injury. How to cognize the objective authenticity on the therapeutic and toxic effects of rhubarb? In other words, how to reveal the dosage-efficacy/toxicity relationship of rhubarb? To answer those questions, we investigated the syndrome (disease)-dosage-toxicity-effect relationship of rhubarb based on the DbTox, i.e., clarifying the relationship and related factors by comparing normal with disease animals (of hepatitis and nephritis models). Aimed to provide scientific reference for rational use of rhubarb, it is also a good paradigm for the syndrome (disease)-dosage-toxicity-effect relationship and therapeutic window research of H&TM.

The results demonstrated that the tolerance and the dosage-toxicity-effect relationship of rhubarb and its preparations had significant differences in rats of different ages or syndromes, such as chronic liver injury or renal failure.^[17-22] Compared with normal rats, the tolerance to rhubarb of the CCl₄-treated rats significantly increased about 2-4 times, and rhubarb showed the dose-dependent therapeutic effects within the safe dose and time range.^[20-22] It protected the liver cells by reducing the cellular injury factor, while it induced liver injury by increasing the fibrosis factor. The rhubarb group within the dosage of 2–14.67 g/kg showed the hepatoprotection and decreased aminotransferases, and that efficacy was significantly dose dependent. Although aminotransferases and other biomarkers of liver function still decreased in 14.67–40 g/kg, the liver fibrosis was observed, which was significantly enhanced with the increase of dosage. Thus, the possible therapeutic window of rhubarb for chronic liver injury is 2–14.67 g/kg (equivalent to 0.3–2.5 g/kg/d for human).^[22] The above results indicated that syndrome differentiation-based toxicity attenuation of rhubarb could be performed by applying in right indications, dosage, and medication cycle. Metabolomic study demonstrated that the liver, kidney, and spleen tissue distributions of rhubarb anthraquinone derivatives (e.g., rhein, aloe-emodin, and emodin) in normal rats were higher than those on CCl₄-treated models, which demonstrated significant correlation with the bidirectional effects of rhubarb, both liver protection and hepatotoxicity,^[23,24] and revealed the possible mechanisms of the You Gu Wu Yun phenomenon. It is consistent with the content of Records of Traditional Chinese and Western Medicine in combination

written by Xichun Zhang (Qing dynasty) that though the property of rhubarb is fierce if it is used on right patients, the disease could be cure, even some time to take larger dosage is acceptable. Meanwhile, it objectively validates the You Gu Wu Yun phenomenon of rhubarb, which means that rhubarb will not cause any injury (Wu Yun) to sick body with liver injury (You Gu) within the certain dosage range by syndrome differentiation-based medication. It well explains the bidirectional effects of rhubarb about toxicity reports and common indications for liver and kidney diseases and provides scientific evidence for the syndrome differentiation-based toxicity attenuation.

Evaluation and risk control for idiosyncratic toxicity of herbal and traditional medicine based on susceptible models

Idiosyncratic toxicity is one of the most challenging problems and hot issues on the safety of H&TM. In recent years, the reports on the liver injury induced by *Polygonum multiflorum* Thunb. (PM) and its preparations have been arising,^[25] which caused domestic and overseas concerns. PM is a representative of conventional deemed “nontoxic” H&TM. Clinical studies demonstrated that it was safe for overwhelming majority of patients to administrate PM, while it would induce liver injury in a small portion of susceptible individuals. It is a typical idiosyncratic hepatotoxicity, and the toxic dosage and course is within the clinical dose range. Nevertheless, it observed by the conventional toxicological methods that PM could induce liver injury on normal experimental animals under the extremely large dosage and long-term treatment. The toxic dosage varies so widely from the clinical situation in patients that the method obviously cannot evaluate the idiosyncratic hepatotoxicity induced by PM accurately.

The previous study confirmed that PM-induced liver injury was more likely to happen among susceptible individuals,^[26-28] and it had a close correlation with the immune status of the body. We investigated with international advanced research on immune and hepatotoxicity,^[29,30] and full considered that PM has strong efficacy to boost immune function, and the components are of potential hepatotoxicity. The immune-mediated model to assess PM-induced liver injury was established,^[31] the Three Factors Causing Toxicity mechanism hypothesis of TM-induced liver injury was put forward, and the relationship between immune stress with susceptibility to PM was elaborated. The Three Factors Causing Toxicity mechanism hypothesis is that when the human’s immunity is abnormal activated (under the susceptible condition), the immunogenic components of PM (such as trans-stilbene glucoside) will further increase the level of immune activation (immunological stress). Meanwhile, the susceptibility to some potential susceptible components (e.g., THSG dimer,^[32] cis-stilbene glycoside,^[33] and emodin-8-O-β-D-glucoside) rises, the overexpression of inflammatory cytokines could induce immune-mediated liver

injury. In other words, it is like the relationship between wood, fuel, and embers. Abnormal immune activation in body, the immunogenic components of PM and potential susceptible components are metaphorically referred to the wood, fuel, and embers, respectively. Any single factor of them will not cause fire, only when three risk factors are combined, little embers will develop to a big fire (intoxication). The immunogenic components of PM will further promote the abnormally activated immune system, and then the potential susceptible components of PM could induce liver injury. The Three Factors Causing Toxicity, which has been proposed and preliminarily validated, closely integrates with the characteristics and risk factors of H&TM-induced liver injury. It fully considers the influence of the individual status on the susceptibility and provides new insight into understanding the idiosyncratic toxicity of conventional deemed “nontoxic” H&TM.

Further analyzing the individual status and syndromes, that abnormal immune activation in most cases of PM-induced liver injury is corresponding to the “blood-heat consuming Yin” or “Yin-deficiency and Yang-hyperactivity” syndrome in the diagnosis of Chinese medicine, with immune activation or increased inflammatory cytokines. While PM has strong efficacy to boost immune function, which can further induce immune activation or immunological stress. Hence, the Three Factors Causing Toxicity Mechanism hypothesis of idiosyncratic hepatotoxicity induced by PM was put forward. Experimental animals validated it, and then, the idiosyncratic hepatotoxicity assessment model of PM associated with immunological stress was established for the first time.^[31] It was found by investigating the dosage-toxicity and the time-toxicity relationship, which the two folds of clinical equivalent dose of PM (12 g/day for human) could result in liver injury in the rat assessment model. The metabolic pathway of TLR4-NF-KB was activated, the inflammatory cells were differentiated and activated, the inflammatory cytokines and chemokines were released, such as TNF-α, IL-1β, IL-6, MIP-2, and MCP-1, the stability of tissue microenvironment was disturbed. Those alternations make the liver more susceptible to medicines, and then, the idiosyncratic hepatotoxicity happens. The toxic dosage of PM in the immunological stress model is consistent with that taking by patients in liver injury cases. In contrast, there is no hepatotoxicity in normal animals with even 70 times that dosage. The results show that the model based on risk factors, which could better evaluate the idiosyncratic hepatotoxicity of PM, provides an important tool for investigating the toxicology mechanism and screening susceptible population.

CONCLUSION AND PROSPECT

Nowadays, the era of precision medicine is taking great pace toward us. To some extent, the notion of personalized diagnosis and treatment in Chinese medicine is the origin of precision medicine. Chinese medicine emphasizes the

importance of syndrome differentiation-based toxicity attenuation and the You Gu Wu Yun, which are the ancient people's understanding and early practice of precision toxicology. Presented as speculation, reasoning, and experience, the cognition on the toxicity of H&TM did not form a theory system, without integrated or scientific explanation. Therefore, it causes the embarrassment that toxicity theories of H&TM cannot be easily accepted by the modern medical and scientific system and that it is difficult to carry out related mechanism research. The DbTox reviews the traditional toxicity theory of syndrome differentiation-based toxicity attenuation from the perspective of modern toxicology for the first time, makes a delicate combination of systematic toxicology, predictive toxicology, and precision medicine. It puts forward the point that the safe use of H&TM partly depends on different individual status, which includes not only different diseases, syndromes, but also metabolic and immune status, habitus, and genetic polymorphisms. The biological basis of those differences is that genes, enzymes, metabolites, and together with tissue microenvironments of people are different. The DbTox provides theoretical and systematic guidance for exploring the mechanism of H&TM toxicology from the cellular and molecular level, and gives an approach to the translation of traditional theories from subjective speculation to objective evaluation.

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Conflicts of interest

There are no conflicts of interest.

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