Review Article

Discussions on Toxic Traditional Chinese Medicine and New Perspectives

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Abstract

Along with the increase in the consumption of traditional Chinese medicine (TCM), the safety of TCM has dramatically attracted the attention and concern of the public. Here, we review previous studies, which focused mainly on the toxicity of toxic TCM and the interpretations for combination, to elaborate on advances and important issues existing in the safety evaluation of TCM, aiming to provide scientific advice for the clinical use. Moreover, we emphasize the importance of a safe evaluation system for TCM based on the material basis for toxicity, which integrates new toxicity testing strategy and is launched under the guidance of TCM theories in future researches.

Keywords: New toxicity testing strategy, safety, toxicity mechanism, traditional Chinese medicine

Introduction

Traditional Chinese medicine (TCM) is not only an important vehicle of Chinese civilization but also plays a unique role in public health and contributes enormously to the reproduction and health of the Chinese nation and some areas of Asia for thousands of years.[1] At present, more and more countries or areas are prone to take TCM as a complementary health approach or dietary supplements.[2,3] Since the early 20th century, the adverse reactions of TCM have gradually attracted the attention of the public, and growing studies began to explore the safety of TCM. Comparing with modern Western medicine, Chinese medicine has unique theoretical basis and philosophical basis. Therefore, the concept of "adverse reactions" in modern medicine could not be simply defined as "toxicity" of TCM. Nowadays, there is a general phenomenon that people confuse the concept of "toxicity" between TCM and modern medicine. The "toxicity" for TCM has two implications. For one, it refers to the characteristic of TCM, which is inherently in the TCM. Another is related to the toxic effects on the human body. This kind of TCM is normally poisonous and can produce toxic side effects, so it is also called "toxic TCM." However, classic theories have also indicated that appropriate administration of these toxic TCMs could generate obvious therapeutic effects under specific pathological status. Hence, our team focused on the safety evaluation of these TCMs using modern scientific technologies and toxic mechanisms underlying these TCM-induced adverse effects, in order to provide solid and reliable basis for the proper use of TCM and offer better suggestions to their clinical applications.

Based on the theory of TCM toxicity, three critical problems must be resolved in the related researches. First, the material basis for the toxicity of TCM (toxic components), the relationship between toxic components and pharmacological components, and the toxicity–efficacy characteristics of toxic TCM should be scientifically descripted. Second, the toxicity of toxic TCM and molecular mechanisms involved will be systematically investigated based on toxicological effect, molecular mechanism, and signaling pathway levels. Finally, new thinking for scientific safety evaluation of toxic TCM should be raised. Next, we would review previous studies on toxic TCM from these three fields,

Access this article online

Quick Response Code:

Website: www.wjtcm.net

DOI: 10.4103/wjtcm.wjtcm_27_19

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Received: 31-05-2019, **Accepted:** 19-08-2019

How to cite this article: Liu H, Zhou W, Gao Y. Discussions on toxic traditional Chinese medicine and new perspectives. World J Tradit Chin Med 2019;5:145-50.

to present the mechanisms of TCM toxicity, *in vivo* metabolic processes or toxicokinetics characteristics of toxic components, and new toxicity testing strategy for toxic TCM.

Toxic Effects and Mechanisms of Toxic Traditional Chinese Medicine

Hepatotoxicity

As the primary metabolic organ, the liver is more sensitive to toxic reactions than other organs when the body is exposed to exogenous chemicals. The hepatotoxicity of TCM has become a safety issue attracting the bulk of attention worldwide.[4] TCM with hepatotoxicity, such as *Polygonum multiflorum* (PM),[5] *Fructus Meliae Toosendan* (FMT),[6] *Fructus Psoraleae* (FP),[7] and *Panax notoginseng*,[8] have been well documented.

PM is applied widely to the treatment of alopecia, hypertension, and hyperlipidemia.[9,10] It is generally believed that the components of anthraquinone such as emodin and rhein are the main substances leading to liver injury. Previous studies on the mechanism underlying emodin-induced liver injury show that emodin significantly triggers the mRNA and the protein expression of liver drug enzymes such as CYP1A1 and CYP1B. Moreover, in the condition of inhibition of CYP1A1 or AhR, the cytotoxic effect of emodin decreased obviously. Hence, PM causes liver toxicity possibly by inducing the perturbations in enzyme activity.[11]

FMT, always used as an insecticide against the intestinal parasite, has been reported to cause obvious hepatic injury.[12] In the liver of mice, the expression profiles of mRNA and miRNA after the administration of the FMT extraction using ethyl acetate were analyzed by Ji *et al*.,[13] and they found that lipid disturbance plays a vital role in FMT extraction-induced liver injury. Consistent with another study from Lu *et al*.,[14] FMT-induced hepatic injury was confirmed to be related to abnormal glutathione depletion, mitochondrial dysfunction, and lipid metabolism using general toxicological assessments integrating miRNA–mRNA analysis.

FP is used for the treatment of osteoporosis and some skin diseases.[15] However, some components of FP may produce toxic effects, especially hepatotoxicity. Bavachin, the hepatotoxic substance, is the main flavonoid in FP. Yang *et al*.[16] reported the toxicity induced by bavachin and the possible mechanisms that bavachin could dose dependently trigger cell apoptosis and endoplasmic reticulum (ER) stress, which could be obviously attenuated by cotreatment with tauroursodeoxycholic acid, knocking down mitofusin 2, or inhibiting the phosphorylation of protein kinase B (Akt). Besides, reactive oxygen species (ROS) scavenger (N-acetyl-1-cysteine) also could reduce bavachin-induced hepatotoxicity.

Nephrotoxicity

The kidney is the main excretory organ of the body. Nephrotoxic TCM could produce nephrotoxicity directly or cause renal injury through allergic reaction indirectly. TCM with nephrotoxicity has *Tripterygium wilfordii* (TW),[17,18] *Leonuri herba* (LH),[19] *ephedra*, etc.

TW *Hook.F.* is useful in the treatment of rheumatoid arthritis and autoimmune disease. However, the nephrotoxicity from TW has been frequently reported. A study[18] showed that triptolide (TP) is the key compound of TW, which could produce nephrotoxicity. Qu *et al*.[17] found that oxidative stress in the kidney became more serious and the expression of enzymes correlated with sphingolipid metabolism at the transcriptional level changed, which was because of the high dose of TP.

LH is always used to treat the menstrual irregularities, edema, and oliguria. Alkaloids were the main toxic material basis of LH. Qian[19] found that the cause of nephrotoxicity induced by LH total alkaloids was oxidative damage and endothelial cell injury. Besides, the severity of kidney tissue injury showed dose dependency.

Neurotoxicity

The nervous system regulates the activities of other systems to adapt the internal and external environment changes and ensure normal life. Some components in TCM, including *Veratrum nigrum* L., bupleurotoxin, and *Fructus Aristolochiae*,[20,21] are responsible for the damage of structural damage or neurologic dysfunction.

V. nigrum L. is widely used in the treatment of stroke, epilepsy, and other cerebrovascular diseases. Previous article has reported death cases of mice after the administration of water extract of *V. nigrum L.*, accompanying with 5–10 min convulsion. Wang *et al*.[22] also showed that veratridine (Ver), the toxic and effective constituents of *V. nigrum* L., could cause remarkable changes in mitochondrial membrane potential(MMP) expression, Ca^{2+} concentration, and reactive oxygen species ROS production, and lactate dehydrogenase (LDH) release in SH-SY5Y cell, suggesting prominent roles of cytotoxicity in *V. nigrum* L-induced neurotoxicity.[23]

Bupleurotoxin is the main toxicity compounds of *Bupleurum longiradiatum*. The neurotoxicity study of bupleurotoxin based on metabonomics by Zhang *et al*. has identified that several biomarkers for toxicity indication were closely related to kynurenine pathway. Besides, the mechanisms underlying bupleurotoxin-induced neurotoxicity were also confirmed to be related with the inhibition of gamma-aminobutyric acid (GABA) receptors, which could result in the generation of neuronal excitability, excessive discharge of nerve cells, a large influx of calcium ions, and even the death of nerve cells, suggesting that GABA receptors are the important toxic target of bupleurotoxin.[21]

Cardiotoxicity

Cardiotoxicity means the function and organic alteration in heart tissue, which is precise coordinated. More and more reports about the cardiotoxicity of TCM emerge in recent years, such as Fuzi[24] and Ophiopogonin D'.[25]

Fuzi (*Aconitum carmichaelii* Debx) is commonly used to treat rheumatic pain, angina and even cardiac shock in China and other Asian countries.[26] Fuzi extract-induced

cardiotoxicity has been well documented in previous studies; for instance, Huang *et al*.[27] found that diester-diterpene alkaloids (DDAs), the main toxic components in Fuzi extracts, could activate pregnane X receptor (PXR) and increase expression of cytochrome P450 3A4 (CYP3A4). Conversely, monoester-diterpene alkaloids (MDAs), another class of toxic components, exerted no effects on cardiac tissue. Besides, they explored the mechanisms involved in different extracts induced-cardiotoxicity by metabolomics. Collectively, these results revealed that the water and ethanol extracts of Fuzi cause cardiotoxic effects through activating the PI3K/Akt/mTOR signaling pathway and downstream PXR-CYP3A4 axis to induce cardiomyocyte apoptosis.[28]

MDAs include benzoylaconine, benzoylmesaconine, and benzoylhypaconine. Zhang *et al*.[29] focused on the metabolism of DDAs and MDAs in human intestine microsomes, with comparison of metabolism in human liver microsomes. They found that MDAs, mainly catalyzed by CYP3A, occurred in the basic form of dehydrogenation in human intestinal epithelial cells, leading to polarity increase of metabolites. Ye *et al*.[30] evaluated and compared the cardiotoxicity of DDAs and MDAs on the zebrafish. The results showed that DDAs exhibited serious cardiotoxicities, whereas the toxicity of MDAs was quite weaker and slower than DDAs. In high dose, MDAs were K^+ channel inhibitors instead of Na^+ channel inhibitors and induced the decrease of heart rate, but no toxicity on morphology.

Ophiopogon japonicus is effective in the cure of arrhythmia, myocardial ischemia, and other cardiovascular diseases. Ophiopogonin D' (OPD') is one of the toxic ingredients in *O. japonicus*. Ren *et al*. found that a lower dose of OPD' (5 μmol/L) could significantly inhibit the activity of cardiomyocytes, improve the content of cellular ROS, and facilitate the release of LDH. The mitochondrial membrane potential decreased and even disappeared after OPD' treatment, and the intracellular ER stress was also triggered by OPD'-induced overload of intracellular calcium ions, both of which eventually result in OPD'-induced apoptosis.[25]

The Chinese medicine, the toxic components and the toxicity mechanism were showed into Table 1.

Study on Combination (配伍)

Following the drug pair combination, the chemical components of different TCMs proceed a series of complex reactions to increase the dissolution rate of effective components and reduce the dissolution rate of toxic components, so that the side and toxicity effects were weaker while therapeutic efficacy was greater. Besides, it may produce some new ingredients which could expand the treatment scope. Therefore, we can control different proportions of compatibility and detect and analyze the contents of effective and toxic components before and after compatibility to understand the pharmacological and toxicological mechanism on compatibility.

Radix ginseng and *Veratrum nigrum* L.

The combination of TCM used in clinical practices aims to enhance therapeutic efficacy or reduce toxic effects. Eighteen antagonisms ("十八反") are one of the crucial theories for prohibited combination ("配伍禁忌") of TCM. *V. nigrum* L., with obvious cardiotoxicity and neurotoxicity, is used discreetly in a compound formula containing *Radix ginseng*. For ginsenoside, the main component in *R. ginseng* facilitates the dissolution of toxic *V. nigrum* L. alkaloids. This is why that the higher dose of *R. ginseng* used in formula, the more amounts of toxic alkaloids detected in decoction, following a significantly enhance in the cardiotoxicity of *V. nigrum* L.[31] Rats administered *V. nigrum L: R ginseng* ratio of 1:2.63 for

Table 1: The toxicity characteristics of different traditional Chinese medicine

	Traditional Chinese Medicine	The toxic components	Toxicity	Mechanism
1	*Polygonum multiflorum*[11]	Emodin	Hepatotoxicity	Induce the perturbations in enzyme activity
2	*Fructus meliae toosendan*[13]	Toosendanin	Hepatotoxicity	Abnormal GSH depletion, mitochondrial dysfunction, and lipid metabolism
3	*Fructus psoraleae*[16]	Bavachin	Hepatotoxicity	Dose-dependently trigger cell apoptosis and ER stress
4	*Tripterygium wilfordii Hook.F*[17,18]	Triptolide	Nephrotoxicity	Strengthen oxidative stress and changed the expression of enzymes at the transcriptional level
5	*Leonuri Herba*[19]	The total alkaloids of Leonuri Herba	Nephrotoxicity	Induce oxidative damage and endothelial cell injury
6	*Veratrum nigrum L.*[22,23]	Veratridine	Neurotoxicity	Change the expression of MMP, Ca^{2+}concentration, ROS production, and LDH release
7	*Bupleurum longiradiatum*[21]	Bupleurotoxin	Neurotoxicity	Inhibit GABA receptors, result in the generation of neuronal excitability, excessive discharge of nerve cells, and a large influx of calcium ions
8	*Aconitum carmichaelii Debx*[27,28]	Diester-diterpene alkaloids	Cardiotoxicity	Active PXR and increase cytochrome P450 3A4 (CYP3A4) expression: Activate the PI3K/Akt/mTOR signaling pathway and downstream PXR-CYP3A4 axis
9	*Ophiopogon japonicus*[25]	Ophiopogonin D′	Cardiotoxicity	Inhibit the activity of cardiomyocytes, improve the content of cellular ROS, and facilitate the release of LDH

GSH: Glutathione, ER: Endoplasmic reticulum, PXR: Pregnane X receptor, LDH: Lactate dehydrogenase, MMP: Mitochondrial membrane potential, GABA: Gamma-aminobutyric acid (γ-aminobutyric acid), ROS: Reactive oxygen species

8 weeks, Sun *et al*.[32] found that the expression of enzymes related to protein folding and amino acid metabolism in rats was affected.

Radix ginseng and Fuzi

Shenfu injection is mainly composed of *R. ginseng* and Fuzi and is commonly used in the treatment for heart failure by strengthening myocardial contractility and reducing myocardial oxygen consumption. Ma *et al*.[33] used ultra-high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UPLC-TOF/MS) to study chemical constitutions in Shenfu injection. They found that the contents of diester-diterpenoid alkaloids (DDAs) reduced obviously, whereas that of monoester-diterpenoid alkaloids (MDAs) increased, suggesting that ginseng probably attenuates the toxicity of Fuzi by promoting the transform of high toxic DDAs to low toxic MDAs. Moreover, they also pointed that the combination of *R. ginseng* and Fuzi at a ratio of 1:1 could be applied in clinical practices.[34]

Radix ginseng and *Radix ophiopogonis*

Shenmai decoction, which consists of *R. ginseng* and *Radix ophiopogonis*, functions in antioxidation and antimyocardial ischemia by reducing myocardial oxygen consumption and is usually used for the treatment of cardiovascular diseases. Zhou *et al*.[35] found that eight constituents altered markedly in the codecoction of *R. ginseng* and *R. ophiopogonis* using an UPLC-TOF-MS assay. The content of ginsenoside Re, ginsenoside Rg1, and malonyl ginsenoside Rb1 diminished, whereas the content of ginsenoside Rb3, malonyl ginsenoside Rc, and malonyl ginsenoside Rb2, ginsenoside Rf raised. This may be the material foundation of reducing toxicity and increasing therapeutic effect in combination of *R. ginseng* and *R. ophiopogonis*.

Radix paeoniae alba and *Veratrum nigrum* L.

V. nigrum L. and *Radix paeoniae alba* (RPA) have been documented in prohibited combination for thousands of years. Zhang *et al*.[20] found that the mortality of mice was higher with the increased consumption of veratridine and lower with the increased consumption of RPA.[36] The methods of UPLC-Q-TOF/MS and metabonomics were performed to quantify the toxic component substances in *V. nigrum* L. and RPA combination and found that toxic *Veratrum* alkaloids dissolved well with a lower dose of RPA. Hence, a higher dose of RPA could maximize the neuroprotective effects, which could ameliorate the toxicity caused by the combination of *V. nigrum* L. and RPA.

New Perspectives on Safety Evaluation of Traditional Chinese Medicine

New toxicity testing strategies

As the development of modern medicine and biology, there are many animal experiments dominated toxicity testing methodologies in the safety evaluation for TCM,[37] which could give a glimpse into possible toxicity of toxic TCM before clinical test (e.g., the toxic dose and/or the toxicity mechanism) by demonstrating their absorption, distribution, metabolism, and excretion in animals treated with overdose. However, the results obtained from the conventional animal experiments have two obvious shortcomings: first, this conventional strategy is characterized with the lower efficiency and the higher expenses, which is not competent in the evaluation of abundant chemical compounds. Besides, data gaps and extrapolation across species are an impenetrable barrier around assessing toxicological effects of TCM. Facing these problems and challenges, the National Research Council of the United States published a landmark report, Toxicity texting in the 21st Century: a vision and a strategy (TT21C).[38] The report gives a new framework for toxicity testing and risk assessment. One of the elements is the dose–effect and extrapolation model, which includes the physiological-based pharmacokinetic model and the toxic pathway dose–response model using human cells. These models can scientifically extrapolate the exposure dose *in vivo* from the concentration obtained *in vitro*.

Moreover, conventional toxicity assessment prefers to be conducted at extremely high doses that much more than the real-world administration dose in a shorter term to assess the toxicity of TCM. Consequently, conventional toxicity studies for TCM indeed did not perform well in toxicity identification and showed remarkable deficiency in early toxicity screening, molecular toxicology researches, and the development of the dose–effect and extrapolation model for TT21C.[39] Fortunately, incorporating a tiered approach based on real-world exposure scenario to characterized drugs/toxicant-induced cellular response pathway in an exposure-led framework for this next-generation risk assessment (NGRA) enables an understanding of the relevance of tipping points for adoptive/adverse responses.[40-42] Therefore, toxicity studies for TCM based on NGRA will not only contribute to the systematic and precise elaboration of related molecular toxicity mechanisms but also do a favor for the potential toxicity identification and scientific clinical use of these toxic TCMs.[43] At present, there is little report about the application progress of risk assessment in TCM, which will be a new orientation for the evaluation of Chinese medicine safety.

New Technologies

In recent years, Omics such as genomics, proteomics, and metabolomics are also a hotspot fascinating many toxicologists, who have imported this technology into their own researches and constructed a genetically engineered drug toxicity screening model *in vitro* and *in vivo*, and provide powerful theoretical and methodological support for comprehensive and systematic toxicity evaluation.[44]

Toxicogenomics

Toxicogenomics mainly focuses on the alterations in gene expression by comparing and analyzing the characteristic expression profiles of cells, tissues, or organs after TCM treatment, combining with available analysis software and gene

database for the target prediction and pattern recognition. It also facilitates the exploration of toxic mechanisms and toxic biomarkers for toxic TCM.[45] For example, Wang *et al*.[22] used gene microarray analysis to screen target genes related to the toxicity of veratridine and presented new thinking for the study on neurotoxicity mechanism of veratridine. Furthermore, based on toxicogenomics, the high-throughput detection and the integration analysis from multiple molecular levels such as microRNA and mRNA is conduced to understand comprehensively the interaction between these molecules in the toxicity development of TCM (extracts or components). Our previous work took FMT[13,14,46] as the research object and revealed the material basis for hepatotoxicity of FMT and the mechanisms underlying by integrating miRNA- and mRNA-based toxicogenomics data on medicinal materials, components, and monomer level.

Proteomics and metabolomics

Comparing with traditional pathological endpoints, the protein markers relating with toxic effects are more sensitive and could be detected at lower doses in a shorter term. Like toxicogenomics, proteomics offers quick protein expression profiles after TCM administration and digs specific proteins related to the toxicity. Then, determining some protein markers is the toxicity indication of TCM by experiments.[47] Besides, metabolomics has its advantage in the demonstration of target tissues of toxicity, toxicokinetics of toxic components, and toxic biomarkers by measuring the dynamic alteration in metabolite profiles in future safety evaluation of TCM. Because the two techniques can provide complete information about the model of action of TCM during the entire process, and display a better consistency and predictability than that detected at a single time-point. More importantly, metabolic modification revealed by proteomics and metabolomics is a true toxicity endpoint instead of a change in gene or protein expression, which is possibly an adverse outcome pathway involved. Hence, the two techniques used in the toxicity assessment of TCM provide convenience to the analysis of toxicity mechanisms and interpretation for efficacy enhancing and toxicity reducing of TCM combination. For instance, Hu *et al*.[48] performed UPLC-Q-TOF-MS method to analyze the effect of water extract of psoralen on endogenous metabolites in rats. After treatment with the water extract of psoralen for 2 weeks, the endogenous substances in the serum of rats were significantly changed, and the mechanism of pharmacological or toxic effects of the psoralen water extract could be well explained from the regulation of amino acid metabolism, phospholipid metabolism, and glycolysis process.

Conclusion

"Toxicity" refers to the inherent characteristics ("偏性") of TCM. In modern Western medicine, each drug could be considered as poisonous. Dosage is crucial for TCM. It treats some diseases efficiently when used properly; otherwise, it could bring some damage. If the dose is lower or higher, it may produce less therapeutic efficacy or toxicity. Although some toxic TCM with over normal dose was used in some cases without toxic reactions, it was specific and could not be applied in everyone. Someone knows insufficiently and misunderstands about TCM because they only see the side of its toxic and side effects. In order to use toxic TCM correctly, we should focused more on processing attenuation (炮制减毒), prohibited combination (配伍禁忌) and the dose-time-treatment-toxicity relationship.

Fully understanding the potential risk of toxic TCM not only avoid frequent occurrence of TCM-induced adverse effects, but also better the process of internalization of TCM. To achieve this goal, it is necessary to carry out system and scientific analysis on the toxic effects and underlying mechanisms of toxic TCM and present rationale combination for toxicity reducing and efficacy enhancing. However, detects existing in the current safety evaluation system for TCM cause improper risk assessment for TCM, especially under the condition without consideration of TCM's real exposure model. Fortunately, new techniques and innovative strategies provide a new version for the toxicity testing of toxic TCM, namely advances in toxicity testing strategy integrating new techniques (such as "-omics" techniques) could play a critical role in reformation and innovation of safety evaluation of TCM in future.

Financial support and sponsorship

This work is financially supported by the National Natural Science Foundation of China (No. 81630102 and No. 81803833).

Conflicts of interest

There are no conflicts of interest.

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