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Review:

Early metabolism evaluation making traditional Chinese medicine effective and safe therapeutics*

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Abstract: Increasing attention is being paid to the scientific evaluation of traditional Chinese medicine (TCM). As many TCMs are capable of biotransformation in the gastrointestinal tract, attention to biotransformation of TCM in the gastrointestinal tract may lead to discovery of the active components and active mechanisms. In this article, we review reports that host metabolic enzymes and intestinal bacteria may be responsible for the metabolism of TCM. Good understanding of the in vivo course of TCM will help us to know how to conduct metabolism evaluation of TCM by using in vitro human-derived system. This evaluation system will create new views on TCM as effective and safe therapeutic agents.

Key words: Traditional Chinese medicine (TCM), Metabolism, Gastrointestinal biotransformation
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INTRODUCTION

There are abundant resources of traditional Chinese medicines (TCMs), which have been clinically used for more than 5000 years, in China and Asia. As TCM is attractive as a pool chemical compounds and natural products for medicinal use, increasing attention is being paid to the scientific evaluation of TCM. However, the study is full of various challenges: varied resource, unknown active ingredients, difficulties in quality control, lack of safety evaluation, or unclear biological mechanism, etc. Due to historical or cultural reasons, most of TCMs have been used as health food or drug without strict experimental evidences or scientific instruction (de Smet, 2002). The US Food and Drug Administration (FDA) has regulations on botanic medicines examination and approval for use in the United States. Although study is dramatically being developed at the source of TCM, the lack of quality standards together with scarcity of mechanism and safety research has seriously limited further development of TCM in China.

Different from single component of western drug, TCM is a complex system consisting of multiple compounds, which makes scientific evaluation of TCM more difficult. Traditional pharmacokinetics methods cannot lead to discovery of the pharmacokinetics properties of TCM, because of the lack of knowledge on their active components. So minimal effective dose and minimal toxic dose of a certain TCM are completely derived from clinical experiences or ancient books, which directly resulted in a number of adverse reactions associated with the use of TCM (Ko, 2004; Siow *et al*., 2005). In addition, traditional pharmacokinetics methods using animal models in drug discovery and development may not be suitable for TCM, although TCM has been clinically used for thousands of years. Species difference

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cannot be excluded by these methods. Therefore, a human-derived evaluation system is urgently needed in the development of TCM, by which quantitive and accurate evaluation of pharmacokinetics properties of TCM can be achieved.

In the last decades, major advances in chemistry, molecular biology, and high-throughput technology have provided rapid and efficient identification of leads in western drug discovery (Kennedy, 1997), but the high attrition rate of candidates (about 90%) in clinical studies has made the pharmaceutical industry realize that they urgently need to increase productivity and development efficiency, and also implement strategies to avoid predictable failure (Eddershaw *et al*., 2000; Prentis *et al*., 1988). Since the 1990's, computer techniques and in vitro techniques using human-derived system, which can characterize chemicals' absorption, distribution, metabolism, and elimination (ADME) properties in the early stage of drug discovery, have dramatically reduced the attrition rate of drug candidates (Kola and Landis, 2004). Therefore, following successful experiences in western and modern drug discovery and development, introducing ADME in vitro techniques using human-derived system into TCM scientific evaluation will enormously accelerate this process. In addition, many TCMs can undergo biotransformation in the gastrointestinal tract. Integration of biotransformation in the gastrointestinal tract into ADME evaluation of TCM will favorably enable discovery of the active components and active mechanisms, and can predict the role of their metabolites in the early stage of TCM evaluation. In the present article, we review reports on how to conduct early metabolism evaluation of TCM by using in vitro ADME-based human-derived systems.

IN VIVO COURSE OF TCM

Different from the single component of western drugs, TCM is a complex system consisting of multiple compounds. Degradation of TCM in the gastrointestinal tract is often observed (Kobashi *et al*., 1992; Kobashi and Akao, 1997; Hasegawa, 2004; Dreessen *et al*., 1981; Shu *et al*., 1987). Therefore, good understanding of TCM metabolism is based on a good understanding of the in vivo course of TCM.

As TCMs are orally administered in most cases, the gastrointestinal tract serves principally as absorption site for absorption and first biotransformation site. TCM biotransformation in the human gastrointestinal tract includes degradation by acid or intestinal bacteria in gastrointestinal tract, and the biotransformation by metabolic enzymes in the intestinal epithelium. Both Phase I and Phase II metabolic enzymes are expressed in the human gastrointestinal tract, together with associated transporters (Kaminsky and Zhang, 2003). The transporters, despite their importance for the fate of enterocyte-absorbed xenobiotics, are beyond the scope of this mini review.

The naturally occurring components of TCM and/or their degradation products in the gastrointestinal tract might be metabolized by metabolic enzymes in the intestinal epithelium, and/or influence their activities. After the TCM components are absorbed into the bloodstream through the intestinal epithelium, they are first delivered to the liver via the portal vein. A substrate of metabolic enzymes can be effectively cleared by intestinal or hepatic metabolism before it reaches the systemic circulation, a process known as first-pass metabolism. In liver, most absorbed components lose their activities, although their metabolism can also sometimes lead to the generation of active metabolites, which can be solely or partially responsible for the pharmacological response. In some instances, metabolism can also produce reactive or toxic intermediates or metabolites, with potential toxicological implications. The components reaching the liver and their metabolites might also possibly influence the activities of metabolic enzymes.

So good understanding of TCM metabolism needs good understanding of the following information (Fig.1):

1. Quantitative content of main components;
2. Degradation course in human gastrointestinal tract (intestinal metabolite profile);
3. Role of metabolic enzyme in intestinal epithelium responsible for their biotransformation, as well as metabolic stability and metabolite profile;
4. Influence naturally occurring TCM components and/or their degradation products on intestinal metabolic enzymes;
5. Identification of components that can be absorbed from the gastrointestinal tract;
6. Biotransformation in the liver of the components that can be absorbed into the bloodstream;

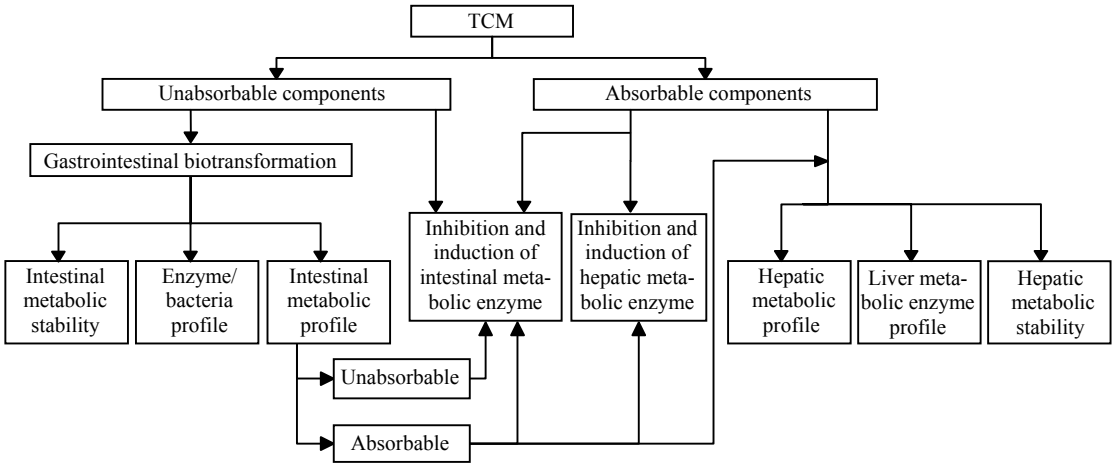

Fig.1 The in vitro characterization of TCM metabolism

7. TCM components' influence on hepatic metabolic enzymes that can be absorbed into the bloodstream;

8. Biotransformation potential in gastrointestinal tract (intestinal bacteria, acid, or intestinal metabolic enzymes) and liver (hepatic metabolic enzymes).

IN VITRO CHARACTERIZATION OF TCM METABOLISM

Intestinal biotransformation of TCM

The gastrointestinal tract is the first site of biotransformation for orally administered xenobiotics or TCM; although the role of gastrointestinal biotransformation is still not being paid its deserved attention to current studies. Gastrointestinal microorganisms comprise an important component of the diverse and dynamic intestinal ecosystem. There are approximately 10^{12} parenchymal cells in an average human (excluding blood cells and neurons), but about 10^{12} bacteria on the skin, another 10^{10} in the mouth, and the gut contains 10^{14} microorganisms (weighing>1 kg) (Mackie *et al*., 1999; Hooper *et al*., 2001). The main human intestinal bacteria are predominantly obligate anaerobes and include species of the genera *Bacteroides*, *Clostridium*, *Lactobacillus*, *Escherichia* and *Bifidobacterium*, together with various yeasts and microorganisms coexisting in dynamic ecological equilibrium. The estimated more than 1000 species of gut microorganisms presents an important challenge to understanding the microbiotal contribution to drug/xenobiotic metabolism and the development of human disease (Nicholson *et al*., 2005).

TCM degradation in gastrointestinal tract is often observed. Kobashi *et al*. proposed the concept of plant glycoside acting as a "Bioprodrug" metabolized to the active form by intestinal bacterial deglycosylation (Kobashi *et al*., 1992; Kobashi and Akao, 1997).

The main metabolic pathways of naturally occurring ginsenosides in gastrointestinal tract are supposed to be as follows: protopanaxadiol-type, Rb_1 →Rd→F_2/M9→M13→C-K (M1), Rb_2→M6→M2→C-K, Rc→M7→M3→C-K, (C-K is gradually hydrolyzed to M12 (Ppd)); protopanaxatriol-type, Re→Rg_1→F_1/Rh_1→Ppt (M4), Rg_1→F_1/Rh_1→Ppt (Hasegawa, 2004). There are also instances of TCM biotransformation by intestinal bacteria, such as sennosides (Dreessen *et al*., 1981), paeoniflorin (Shu *et al*., 1987), baicalin, glycyrrhizin, geniposide (Yim *et al*., 2004), and by flavenoids (Kim *et al*., 1998).

Clarifying the active ingredients of TCM

Knowledge of the in vivo course of TCM is essential for clarifying the active mechanism. According to classic theory of drug action, the concentration of a drug available to bind a target receptor is that which is free in solution within the target organ. So the active ingredients of TCM should be those that can enter the systemic circulation, and include the naturally occurring components and their gastrointestinal metabolites produced by intestinal bacteria or other gastrointestinal factors. The same theory should hold true for the effect of TCM components on

metabolic enzymes (Margolis and Obach, 2003). In addition, TCM components which cannot be absorbed from the gastrointestinal tract, could also possibly act solely or cooperatively on some targets in the gastrointestinal tract, such as enzymes or transporters. Therefore, clarifying the active ingredients of TCM is the primary issue in TCM metabolism research.

Although sennosides have no jalap activity, their metabolites, particularly rheinanthron in the intestinal microflora exhibit potent function of promoting fecal excretion (Dreessen *et al*., 1981). Yang *et al*.(1996) purified and characterized the sennoside-hydrolyzing β-glucosidase from *Bifidobacterium* sp. strain SEN, a human intestinal anaerobe. Oral absorption of paeoniflorin is poor, but paeonimetaboline I formed by human intestinal bacteria exhibited potent anticonvulsant activity (Shu *et al*., 1987; Abdel-Hafez *et al*., 1998). Most naturally occurring ginsenosides including Rb_1, Rb_2, and Rg_1 are poorly absorbed, although their intestinal metabolites including Compound K (C-K), protopanaxadiol (Ppd), and protopanaxadiol (Ppt), are easily absorbed and appear in the plasma of rat or human after oral administration of Rb_1, Re, or Rg_1 (Hasegawa, 2004).

Solubility and permeability assays of components, together with ex vivo culture of intestinal bacteria, purification or in vitro recombination of their metabolic enzymes have become a powerful tool for identifying active ingredients of TCM (Holzapfel *et al*., 1998).

Metabolism of TCM in intestine and liver

Metabolism of drugs represents a key process by which drugs are cleared from the body. They are mainly eliminated by cytochrome P450 (CYPs or P450) enzymes (Phase I metabolism) and, after that, by conjugating enzymes (Phase II metabolism), such as UDP-glucuronosyl transferases and N-acetyl transferases (Delaforge, 1998; Wrighton and Stevens, 1992; Kaminsky and Zhang, 2003). These enzymes add functional groups to make lipophilic molecules more hydrophilic and hence easier to eliminate.

Among hepatic enzymes, the superfamily of CYPs plays a key role in the metabolism of a wide variety of xenobiotics, such as drugs, pesticides and (pre)carcinogens (Wrighton and Stevens, 1992; Gonzalez, 1989). In liver, the most important CYPs from the viewpoint of drug metabolism are CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, which represent about 70% of the total CYPs enzymes and are responsible for the oxidation of more than 90% clinical drugs (Shimada *et al*., 1994; Lasker *et al*., 1998). In intestine, CYP3A4 is the predominant CYP, while only a very limited number of other CYPs including CYP2C9, CYP2C19, and CYP1A1 are expressed (Zhang *et al*., 1999; Obach *et al*., 2001). Intestinal CYPs also have been shown to contribute significantly to the metabolism of several drugs, including nifedipine and midazolam (Holtbecker *et al*., 1996; Paine *et al*., 1996).

Using in vitro experimental system, such as cDNA-expressed metabolic enzymes, microsomes, tissue slice, hepatocyte, or S9, etc., the methods for characterizing the metabolic stability, metabolite profile, metabolic enzyme profile, inhibition or induction of metabolic enzymes have been well established (Gunaratna, 2000).

Metabolic stability

Metabolic stability is one of several major determinants in defining the oral bioavailability and systemic clearance of a drug. The metabolism of both host and intestinal bacteria play an important role in a number of TCMs.

The stability of active TCM ingredients in metabolism by liver and extrahepatic tissues as well as intestinal bacteria will ultimately determine the concentration of the compound found in the systemic circulation and affect its half-life and residence time within the body (Masimirembwa *et al*., 2001; Yan and Caldwell, 2001). By determining the time and concentration dependence of metabolite formation from an active ingredient in vitro and the kinetics properties in an appropriate system, such as intestinal bacteria, microsomes, hepatocytes, or cDNA-expressed metabolizing enzymes, the active ingredient's metabolic fate and half-life in vivo can be predicted. Similar studies on human and test species give valuable information for selecting test species for pharmacokinetics in vivo studies.

Metabolite profile

Metabolite profile is important for the identification of TCM active ingredients. The components that can enter the systemic circulation after oral administration of TCM might be parent compound, metabolic

enzymes-based, or gastrointestinal degradation-based metabolites. Today, there are effective methods for metabolite identification and subsequent construction of metabolic routes. Metabolite identification can be developed from incubations with human liver or intestine preparations (Rodrigues and Wong, 1997; Ferrini *et al*., 1997; Lin and Lu, 1998; Crespi and Miller, 1999). The rapid development of analytical methods, such as liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), etc., makes it possible to determine with high accuracy the exact molecular masses and metabolite structures (Ansede and Thakker, 2004). Similar studies on human and test species yielded valuable information for the selection of test species for toxicological in vivo studies (Chauret *et al*., 1997). In addition, for prodrug needing the activation of metabolic enzymes or gastrointestinal degradation, the research on metabolite profile is also vital (Delaforge, 1998).

Metabolic enzyme profile

Metabolic enzyme profile includes both the enzymes of host and those excreted by intestinal bacteria, which are involved in the metabolism of TCM. The content and activity of CYPs exhibit a high degree of inter- and intra-individual variability (Shimada *et al*., 1994; Iyer and Sinz, 1999). The genetic polymorphism of CYPs is extensive, and the rate of metabolism for a certain drug can even differ 1000-fold between phenotypes (Ingelman-Sundberg, 2004). In addition, human intestinal bacteria exhibit a high degree of intra-individual variability, which is very changeable depending on host conditions, including diet, health, and even stress. Bacterial ginsenoside-hydrolyzing potentials also exhibit a high degree of inter-individual variability in humans and experimental mice (Hasegawa, 2004). There might be tremendous difference in pharmaceutical activities and adverse reactions among patients administered the same dose of TCM (Schaeffeler *et al*., 2003). Therefore, the identification of the metabolizing enzyme profile will be of help to clarify the active mechanism and determine the direction of individual medicine.

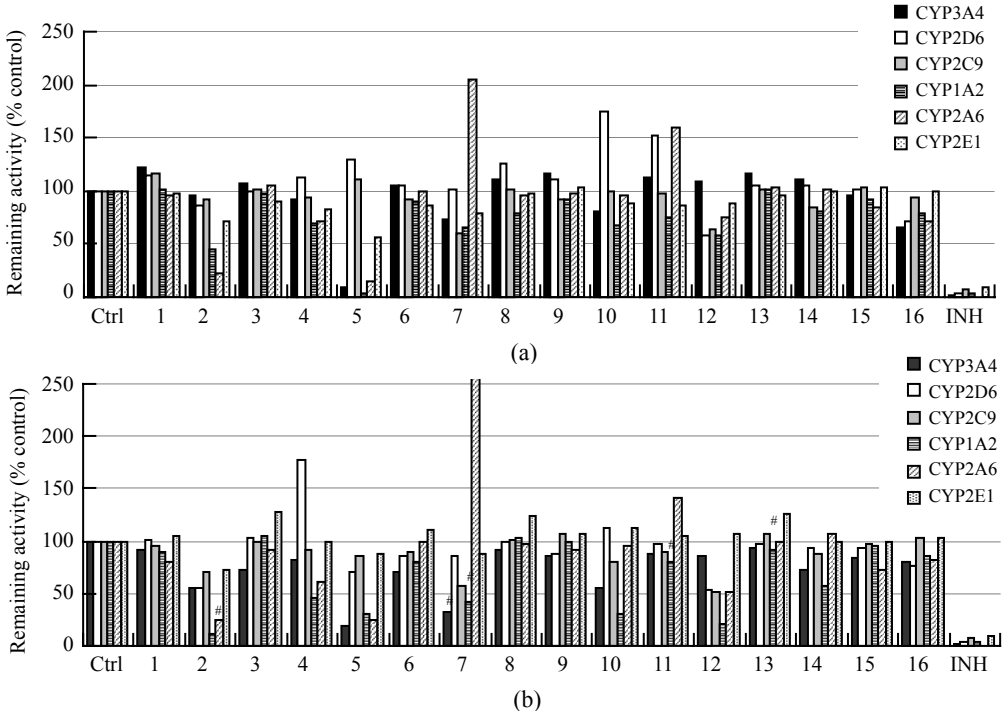

Fig.2 The contrast of inhibition on main cytochrome P450 isoforms of TCM extracts (a) and their degradation products by snailase (b) in human liver microsomes

1: Rizome of Chuanxiong Ligusticum; 2: Dan-shen; 3: Safflower; 4: Radix Paeoniae Rubra; 5: Dalbergia wood; 6: Siberian Thorowax; 7: Fruit of Wilson Citron; 8: Sanchi; 9: Rhizome of grassleaf sweetflag; 10: Immature fruit of Trifoliate-orange; 11: Cassia twig; 12: Spine of Chinese Honeylocust; 13: Seed of Spine Date; 14: Xuefu; 15: Guanxin; 16: Corydalis glaucescens Regel; Ctrl: Negative Control; INH: Positive Control; $^{\#}$ P<0.05 versus parent TCM extracts

Inhibition and induction of metabolic enzyme

It is likely that some of these components share metabolic pathways catalyzed by the same enzymes. In addition, it is most likely that coadministered prescription drugs can be affected by the components of TCM, leading to alterations in their pharmacokinetic profile. This would lead to unwarranted adverse interactions and/or therapeutic failure (Kedderis, 1997). The metabolic enzymes involved include both the enzymes of host and those excreted by intestinal bacteria, which are involved in the metabolism of TCM.

There are a number of reported herb-prescription drug interactions via the influence of metabolizing enzymes, such as St. John's Wort, ginseng, ginkgo, garlic, etc. (Barone *et al*., 2000; Piscitelli *et al*., 2000; Rosenblatt and Mindel, 1997; Sunter, 1991; Yue *et al*., 2000). Up to now, most studies on the influence of TCM on metabolic enzymes focused on their naturally occurring components. However, Fig.2 (in the preceding page) shows that the degradation products of TCM by gastrointestinal factors may exhibit distinct effects on metabolic enzymes compared to naturally occurring components. Liu *et al*.(2004) reported that naturally occurring ginsenosides including Rb_1, Rb_2, Rc, Re, Rg_1 and one of the intestinal metabolites of ginsenosides, C-K, had no inhibitory effect, whereas another intestinal metabolite, Ppt, exhibited potent inhibition against rat CYP3A activity.

The biotransformation of naturally occurring TCM components in the gastrointestinal tract may play an important role in TCM-associated drug interactions via CYPs inhibition.

CONCLUSION

In summary, host metabolic enzymes and intestinal bacteria may be responsible for TCM metabolism. The characterization of TCM metabolism needs a good understanding of the in vivo course of TCM, which will enormously accelerate the process of scientific evaluation of TCM as effective and safe therapeutics.

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