LC–MS–based Metabolomics in Traditional Chinese Medicines Research: Personal Experiences

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ABSTRACT
LC–MS–based metabolomics has being widely used in traditional Chinese medicines (TCMs) research due to the great coverage of mass ranges, high sensitivity to detect metabolites, and no need of sample derivatization. Herein, we reviewed our research on the applications of LC–MS–based metabolomics in TCMs research over the past decade in the following aspects: herbal authentication, determination of herb harvest time, chemical transformation of herbs during post-harvest handlings (sulfur–fumigation and drying), discrimination of raw and processed herbs, chemical transformation of TCMs during preparation, screening endogenous toxic compounds in TCMs, unveiling synergistic mechanisms between small molecules (such as saponins) and polysaccharides in TCMs, revealing synergistic actions of TCMs with chemical drugs, which demonstrated that metabolomics is a superior strategy in TCMs research concerning the holistic perspectives.

Key words
LC–MS; metabolomics; traditional Chinese medicines

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1. Introduction

Traditional Chinese medicines (TCMs) play an important role in maintaining the health of peoples, and become increasingly popular around the world (Commisso et al, 2013; Ouedraogo et al, 2012). Characterized by multi-components and multi-targets, TCMs are used to treat diseases by balancing yin and yang in the human body instead of a single target (Wang et al, 2008). Therefore it is a challenge to find approaches that can holistically evaluate the safety, efficacy, and quality of complex TCMs (Ning et al, 2013).

Metabolomics is a discipline on the detection and intervention-caused variation of global metabolites in biological systems (Commisso et al, 2013; Nicholson and Lindon, 2008; Ning et al, 2013). Depending on an analysis of different sample spectra or jointly with multivariate statistic analysis if needed, metabolomics can be used to identify potential markers and to discover new targets for future therapeutics (Nicholson et al, 2002; Nicholson et al, 2006). The holistic thinking of metabolomics meets the requirements of TCMs in safety, efficacy, and quality evaluation (Li et al, 2015).

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Several techniques have been used in the metabolomics analysis, including NMR (Smolinska et al., 2012; Sas et al., 2015), GC-MS (Sas et al., 2015), LC-MS (Shulaev, 2006), etc. NMR-based metabolomics is highly reproducible at the request of tiny samples without splited or derivates, but weak in sensitivity (Smolinska et al., 2012; Sas et al., 2015). GC-MS-based metabolomics is highly sensitive, reproducible, and specific for volatile metabolites, however, it has a narrow mass range (Sas et al., 2015). LC-MS-based metabolomics, with greater coverage of mass ranges, allows different metabolites detectable with high sensitivity, and no sample derivatization is needed (Shulaev et al., 2006) (Table 1). Thus, LC-MS-based metabolomics is currently the most popular metabolomics approach (Consonni et al., 2016).

There are two normal strategies in metabolomic experiments: targeted and non-targeted analysis (Table 2) (Johnson et al., 2016). The former is to detect or quantify a part of known endogenous or exogenous metabolites precisely. The compounds of interest are required to be known a priori (Wang et al., 2016, Lendoiro et al., 2012). For the latter, as many metabolites as possible are requested to be detected from samples. With multivariate statistic analysis, a holistic chromatography of samples should be provided under investigation and such compounds that uniquely present in one (group) of the samples should be identified (Figure 1) (Patti et al., 2012).

**Table 1** Comparison on different analytical approaches

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Drawbacks</th>
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<tbody>
<tr>
<td>NMR</td>
<td>Highly reproducible and quantitative</td>
<td>Low sensitivity</td>
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<td></td>
<td>Noninvasive and nondestructive for samples</td>
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<tr>
<td>GC-MS</td>
<td>Highly sensitive and specific</td>
<td>Derivatization</td>
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<td></td>
<td>Highly reproducible</td>
<td>Limited mass range</td>
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<td>Existing database</td>
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<tr>
<td>LC-MS</td>
<td>Noninvasive and nondestructive for samples</td>
<td>Requiring preknowledge about samples</td>
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<td></td>
<td>Greater coverage of mass ranges</td>
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<td></td>
<td>Wide array of mass analyzers</td>
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**Table 2** Comparison on targeted and non-targeted metabolomics

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Targeted</td>
<td>Quantitative</td>
<td>Limited number of compounds targeted</td>
</tr>
<tr>
<td>metabolomics</td>
<td>Low limit of detection</td>
<td>Dose not detect compounds that were not targeted</td>
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<td>Non-targeted</td>
<td>Global</td>
<td>Targeted compounds must be available purified for calibration</td>
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<tr>
<td>metabolomics</td>
<td>Highest throughput</td>
<td>Semi-quantitative</td>
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<td></td>
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<td>Majority of peaks are not identifiable</td>
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<td>Difficult informatics</td>
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<td>Medium throughput</td>
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**Figure 1** Summary of LC-MS-based metabolomics workflow in TCMs research
Over the past decade, we have been working on LC-MS-based metabolomics applied for TCMs researches in the following aspects: herbal authentication, determination of herb harvest time, chemical transformation during post-harvest handlings (sulfur-fumigation and drying), discrimination of raw and processed herbs, chemical transformation of TCMs during preparation, screening endogenous toxic compounds in TCMs, unveiling synergistic mechanisms between small molecules and polysaccharides in TCMs, and revealing synergistic actions of TCMs with chemical drugs. Here we reviewed these progresses to demonstrate the superiority of LC-MS-based metabolomics in TCMs researches with holistic perspectives.

2. Personal experiences on LC-MS-based metabolomics in TCMs researches

2.1 Herbal authentication

Chemical components vary in diversity and contents within medicinal plant species. The therapeutic potential of a formula may be altered when replacing one herb with other(s), probably leading to a serious risk to the human health. Due to close taxonomy, it is difficult to precisely distinguish herbs similar in morphology by conventional authentication criteria, such as shape, texture, or odor. Metabolomic technique facilitates the researchers to rapidly identify the characteristic chemical markers of herbs (Chen et al., 2015).

Leaves of *Panax ginseng* C. A. Meyer (LPG) have been documented as a tonic in *Chinese Pharmacopeia* (Pharmacopoeia Committee of P. R. China, 2015). In recent years, the leaves of *Panax quinquefolius* L. (LPQ), similar to LPG in morphology, have been used as the adulterant of LPG, which is unknown in efficacy and safety. So the non-targeted metabolomics coupled with multivariate statistical analysis approach was employed to investigate the metabolic profiles and characteristic chemical markers of herbs (Chen et al., 2015).

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2.2 Determination of herb harvest time

Harvest time may affect the quality of herbs. An LC-MS-based non-targeted metabolomics approach was used to investigate the chemical profile of *Chuanxiong Rhizoma*, a commonly used Chinese medicinal herb. A total of 18 major constituents were identified, i.e. vanillin (1), ferulic acid (2), senkyunolide I (3), senkyunolide H (4), senkyunolide A (5), coniferyl ferulate (6), Z-ligustilide (7), neocnidilide (8), 3-butyldienephthalide (9), senkyunolide J (10), senkyunolide F (11), 3-butyldiphthalide (12), cnidilide (13), riligustilide (14), Z,Z′-6,8,7,3′-diligustilide (15), tokinolide B (16), levistolide A (17), and senkyunolide P (18) (Li et al., 2003), in which nine compounds (compounds 2–6, 7, 9, 14, and 17) are dominated. Then, the nine major components were quantified in *Chuanxiong Rhizoma* samples collected in several growth periods by HPLC-UV-based targeted-metabolomics (Li et al., 2005). The content of each compound gradually increased from the beginning of October to the middle of next April, so did the total ones. As the rhizome keeps increasing in weight until at the end of May, while the total content of the nine compounds peaked in the middle of April, so it is better to harvest *Chuanxiong Rhizoma* from the middle of April to the end of May (Li et al., 2005).

2.3 Chemical transformation of herbs during post-harvest handlings

Post-harvest handling may significantly affect the quality of medicinal herbs. Conventional phytochemical method is time-consuming to evaluate the quality variations of herbs during post-harvest handling. Non-targeted and targeted metabolomics are rapid and reliable platforms for investigating the influence of post-harvest methods on chemical variations in herbs.

2.3.1 Sulfur-fumigation

Since sulfur-fumigation can prevent herbs against insects and molds, it has been widely used for herb processing over the recent decades. For guarantying the efficacy and safety of TCMs, it is very important to know whether the constituents of herbs change or not after fumigated.

*Ginseng Radix et Rhizoma* (ginseng) was reported to be sulfur-fumigated during post-harvested handling. A HUPLC-QTOF-MS/MS-based non-targeted metabolomics approach can rapidly reveal chemical transformation in sulfur-fumigated ginseng (Li et al., 2012). A total of 82 components were detected in non-fumigated and sulfur-fumigated ginseng samples, of which 35 sulfur-containing compounds were detected only in sulfur-fumigated ginseng and its decoction, which were assigned to be sulfate or sulfite derivatives of original ginsenosides. And these sulfur-containing compounds were deduced to be generated via reactions of esterification, addition, hydrolysis and dehydration during sulfur-fumigation and decoction. Then non-fumigated and fumigated ginsengs were compared in the contents of 14 major ginsenosides by an HPLC-UV-based targeted metabolomics (Jin et al., 2012). Nine ginsenosides decreased by almost 54% after sulfur-fumigation. Compared with that of non-fumigated ginseng, the contents of 10 ginsenosides detected in decoctions of sulfur-fumigated ginseng were decreased by about 33%–83%, and the total content of ginsenosides was decreased by up to 64%. In addition, ginsenosides Rh2 and Rg1 could be detected in the decoctions of sulfur-fumigated ginseng but not in non-fumigated ginseng. Furthermore, the targeted-metabolomics strategy showed that the sulfur-containing compound 25-hydroxyl-Re sulfate can be used as chemical...
marker to rapidly screen commercial sulfur-fumigated ginseng (Zhou et al, 2014). Twenty-one commercial ginseng samples from different cities in China were analyzed by the method. It was found that nearly 43% ginseng samples were sulfur-fumigated. The results consist well with that obtained from the method in Chinese Pharmacopoeia 2015. And the proposed approach is clarified to be more rapid and specific for screening sulfur-fumigated ginseng than that in Chinese Pharmacopoeia 2015 (Zhou et al, 2014).

**Paeoniae Alba Radix (PAR)** was reported to be another herb sulfur-fumigated during post-harvest handling. A UPLC-PDA-QTOF-MS/MS based non-targeted metabolomics was established to compare global quality of sulfur-fumigated and non-fumigated PAR (Li et al, 2009). Besides paeoniflorin sulfonate, five more monoterpane glycoside sulfonates were newly identified. These six monoterpane glycoside sulfonates were found characteristic to sulfur-fumigated PAR. Then a targeted-metabolomic method was developed to evaluate the quality of PAR sulfur-fumigated with different durations and purchased from commercial herbal markets (Kong et al, 2014). Twelve major components including paeoniflorin and paeoniflorin sulfonate were analyzed. It was found that paeoniflorin decreased, whereas paeoniflorin sulfonate increased with the prolonged sulfur-fumigation. Paeoniflorin sulfonate was found with varied content in 10 of 17 commercial RPA samples, indicating that 10 samples may be sulfur-fumigated with different durations. Moreover, the relative standard deviation of the contents of each component was higher in the commercial sulfur-fumigated PAR samples than that in commercial non-fumigated ones, and the percentage of total average content of monoterpane glycosides in the determined analytes was higher in the decoctions of sulfur-fumigated PAR than that in non-fumigated RPA samples, suggesting that sulfur-fumigation can not only affect the proportions of bioactive components in RPA, but also interfere the quality consistency of both raw materials and decoctions of RPA (Kong et al, 2014).

Using paeoniflorin sulfonate as a chemical marker, an HPLC-TQ-MS/MS based targeted-metabolomics method was developed to screen commercial sulfur-fumigated PAR in preparations. With multiple reaction monitoring (MRM) scan, i.e., m/z 543→m/z 259 and m/z 543→m/z 213 for paeoniflorin sulfonate, and m/z 449→m/z 327 and m/z 449→m/z 165 for paeoniflorin respectively, the sensitivity and selectivity were tremendously increased compared with the HPLC-UV method (Wu et al, 2012). Seven PAR-containing complex preparations with different constituent herbs were analyzed using the method. It was found that paeoniflorin and paeoniflorin sulfonate were simultaneously detected in all seven PAR-containing complex preparations collected, and no other peaks interfered with the detection of these two compounds even in seven different complex preparations (Wu et al, 2012).

Sulfur-fumigation has also been frequently employed to process Angelicae Sinensis Radix (ASR). A UHPLC-QTOF-MS/MS based non-targeted metabolomic approach was established to identify the characteristic chemical markers for inspecting sulfur-fumigated Angelicae Sinensis Radix (S-ASR) (Bai et al, 2015). (3Z)-6-sulfite-ligustilide and (3E)-6-sulfite-ligustilide were found characteristic to S-ASR. By extraction ion strategy with the typical ion (m/z 271.06) of (3Z)-6-sulfite-ligustilide/(3E)-6-sulfite-ligustilide as the diagnostic ion, another six sulfur-containing components were discovered in S-ASR by targeted metabolomics, such as 6, 8-disulfito-ligustilide and 8-sulfite-ligustilide or their isomers. The generation mechanisms of these compounds were proposed as the addition reaction of sulfurous acid with (Z)-ligustilide and/or (E)-ligustilide, two major components in ASR, at the double bonds of C6-C7 and C3-C8 positions. Using (3Z)-6-sulfite-ligustilide and (3E)-6-sulfite-ligustilide as the chemical markers, 14 of 16 batches of commercial ASR samples were inspected to be S-ASR by targeted metabolomics (Bai et al, 2015).

### 2.3.2 Drying

Drying can also prevent herbs against molds, and prevent biochemical reactions that alter the chemical characteristics of herbs (Ghasemi et al, 2013). There are few studies on influence of drying methods on metabolic profiles of herbs. HPLC-UV based targeted-metabolomic analysis with eight components as chemical markers was developed to evaluate the effects of drying on the quality of Chuanxiong Rhizoma (Li et al, 2007). After dried at 60 °C or under the sun, three major constituents in Chuanxiong Rhizoma, namely senkyunolide A, coniferylferulate and Z-ligustilide, decreased significantly, while ferulic acid, riligustilide and levistolide A increased significantly. Senkyunolide I and senkyunolide H which were not detected in fresh Chuanxiong Rhizoma appeared in dried samples. It was also found that except sun-drying whole rhizomes, the most common and traditional way to dry Chuanxiong Rhizoma, other drying methods, such as heating whole rhizomes or sliced samples at 60 °C in oven and sun-drying sliced samples, might cause the significant alteration of main components, in particular the three major components, in Chuanxiong Rhizoma (Li et al, 2007).

### 2.4 Discrimination of raw and processed herbs

In TCM practice, discrimination of raw and processed herbs is very important for effective use of TCMs. Coupled with multivariate statistic analysis, non-targeted metabolomics is an ideal tool for rapidly finding characteristic chemical markers in qualifying processed herbs, or evaluating the extent of processing.

**Rehmanniae Radix (RR),** derived from the roots of Rehmannia glutinosa Libosch, is one of commonly used herbs in TCMs. Traditionally, RR has been known to “reduce heat in blood, nourish yin and promote the production of body fluids”, and used for treating maculation, nosebleeds, rash, and skin eruptions, while processed RR can “nourish yin and replenish blood, reinforce essence and marrow”, and has been used for treating anemia, diabetes, dizziness,
tinnitus, nocturnal emission and palpitation. They are difficult to be distinguished due to similarity in irregular shapes, a sticky texture, and black in color. They can be rapidly differentiated using a UPLC-QTOF-MS/MS based non-targeted metabolomics along with multivariate statistic analysis, in which potential chemical markers can be identified for the quality control. Leonuride and 5-(6-D-glucopyranosyl-(1→6)-D-glucopyranosylxyomethyl)-2-furan-carboxaldehyde were rapidly found as the most characteristic markers of raw and processed RR, respectively (Li et al, 2010). Furthermore, together with these characteristic chemical markers, eight major components (four iridoid glycosides, three phenethylalcohol glycosides and one furfural derivative) in different batches of raw and processed RR could be simultaneously determined by HPLC-TQ-MS/MS based targeted metabolomics (Xu et al, 2012). The non-targeted metabolomics can be used to compare raw and processed herbs in the global chemical profiles without isolation, purification, and identification (Li et al, 2010a).

### 2.5 Chemical transformation of TCMs during preparations

A TCM formula is usually composed of one or multi-herbs containing multi-components. UPLC-QTOF-MS/MS based metabolomics is a tool for revealing the effects of preparation procedure on quality of TCMs preparations.

Du-Shen-Tang, the decoction of ginseng, is a common prescription as a paragon of emergency treatment in TCM practice. A UPLC-QTOF-MS/MS based non-targeted metabolomics approach was established for quality evaluation of Du-Shen-Tang. A total of 45 major ginsenosides were identified, 21 of which were determined to be produced during decoction. The mechanisms involved were further deduced to be hydrolysis, dehydration, decarboxylation, and addition reactions of the original ginsenosides in ginseng, through analyzing mimic decoctions of 13 pure reference ginsenosides with non-targeted metabolomic strategy (Li et al, 2010b). Then a UHPLC-QTOF-MS based targeted metabolomics method was developed to determine 30 ginsenosides in Du-Shen-Tang decoction and its raw materials derived from *P. ginseng* and *P. quinquefolius*, respectively (Zhou et al, 2014). It was found that the original ginsenosides decreased 25.6% or 7.1%, whereas the degraded ginsenosides and aglycones increased 329.1% or 122.1% in Du-Shen-Tang samples of *P. ginseng* or *P. quinquefolius* compared with their raw materials, indicating that decocting could dramatically increase the proportion of the less polar degraded ginsenosides in Du-Shen-Tang. Since increasing studies showed that ginsenosides or aglycones of less polarity are higher in the bioactivities and the bioavailabilities than the ones of higher polarity (Qi et al, 2010; Wang et al, 2000), the increased proportion of lower polar ginsenosides in ginseng decoction might be responsible for the “emergency treatment” efficacy of Du-Shen-Tang.

Dispensing granule is water extract of individual herb or herbal formula in the form of granule. It is an innovative form of traditional preparation. Due to easier for administration and quality control, the granules are widely used in Southeast Asia in recent years, or even in the United States and some European countries. However, dispensing granule is debated on the efficacy: The dispensing granule may be different from the decoction in chemical components, leading to different efficacy between dispensing granules and traditional decoctions of the same formulae. San-Huang-Xie-Xin-Tang (SHXXT), a formula composed of *Rhei Radix et Rhizoma*, *Coptidis Rhizoma*, and *Scutellariae Radix*, as a model formula, was compared in chemical consistency between traditional decoctions and dispensing granules by a UPLC-QTOF-MS/MS based non-targeted metabolomics coupled with multivariate statistic analysis method (Li et al, 2010b). Thirteen traditional decoction samples and 15 dispensing granule decoction samples were prepared and analyzed. The PCA analysis showed that there was chemical difference between them, in which berberine, palmatine, epiberberine, baicalin, wonogoside, 2-O-gallyl-1-O-cinnamoylgucose, and emodin were identified as the greatest changed components during decocting through OPLS-DA and mass spectra elucidation. Due to significant chemical difference between traditional decoctions and dispensing granules, it is unclear whether or not the traditional decoction of SHXXT can be replaced by dispensing granules. Thus, further comparison in pharmacology, toxicology or even clinic should be investigated (Li et al, 2010b).

Qiong-Yu-Gao (QYG), a classical tonic formula, is composed of *Rehmanniae Radix* (RR), *Poria* (PO) and *Ginseng Radix et Rhizoma* (GR). So far, there are three methods for QYG preparation: Method 1 (M1): mixing powders of GR and PO with RR decoction; Method 2 (M2): the decoction of RR together with PO and then combined with the decoction of GR; Method 3 (M3): decocting the mixtures of RR, GR, and PO. The influence of different methods on the holistic chemical quality of QYG was evaluated by UPLC-QTOF-MS/MS based non-targeted metabolomics. A total of 103 compounds, mainly belonging to ginsenosides, phenethylalcohol glycosides, iridoid glycosides, and triterpenoid acids were identified, of which five degraded ginsenosides were putatively determined to be newly generated during preparation of QYG samples. Triterpenoid acids and malonyl-ginsenosides were detected only in M1 samples, while degraded ginsenosides were merely detectable in M2/M3 samples (Xu et al, 2013). The HPLC-TQ-MS/MS based targeted-metabolomics was developed to quantify 38 major components including the characteristic markers in QYG samples prepared with three different methods (Xu et al, 2015). Programmed ionization mode switching and time segment scanning were designed to simultaneously and more sensitively determine the analytes belonging to five chemical classes, i.e., iridoid glycosides, phenethylalcohol glycosides, furfural derivatives, ginsenosides and triterpenoid acids. Quantitative variations of these components, in particular ginsenosides, degraded ginsenosides and furfural derivatives in samples were
revealed by the method. As mentioned above the degraded ginsenosides are more potent and absorbable than their original ginsenosides. Therefore, different preparation methods may affect the bioactivities of QYG, which needs further investigation.

2.6 Screening endogenous toxic compounds in TCMs

LC-MS-based metabolomics can be used in detecting endogenous toxic compounds in TCMs. Pyrrolizidine alkaloids (PAs), such as those isolated from Senecio scandens Buch.-Ham in India, are known to induce hepatotoxicity and/or tumorigenicity in humans. S. scandens is the botanical plant of Seneciosis Scandentis Herba (Qianliguang) that is one of the major component herbs of Qianbai Biyan Pian, a tablet for treating sinusitis. So it is very important to detect PAs for the safety of Seneciosis Scandentis Herba and Qianbai Biyan Pian. Using the LC-TQ-MS/MS based targeted-metabolomics, nine toxic PAs in S. scandens samples from Shanxi Province of China were identified such as sencenione, sencenionine N-oxide, seneciphylline, seneciphylline N-oxide, senkirkine, jacobine, jacozine N-oxide (or erucifoline N-oxide), usaramine, and an isomer of yamataimine (Li et al, 2008), and they were semi-quantified in contents of 6.95–7.19 μg/g. At a dose equivalent to the daily intake recommended by Chinese Pharmacopoeia 2015, the total content of toxic PAs in Seneciosis Scandentis Herba was determined to be 3.48 μg/kg/d, much less than the minum dose of hepatotoxicity (15 μg/kg/d) suggested by the International Program on Chemical Safety (International Program on Chemistry Safety. http://www.Inchem.Org/documents/ehc/ehc/ehc080.htm). No significant hepatotoxicity was observed in rats fed with Seneciosis Scandentis Herba extract at the human-equivalent dose for 14 consecutive days. These results provided the scientific basis for implementing appropriate dosage guidelines for TCM practitioners (Lin et al, 2009).

2.7 Unveiling synergistic mechanism between small molecules (such as saponins) and polysaccharides in TCMs

Oral decoctions of TCMs serve for therapeutic and prophylactic management of diseases for centuries. Small molecules and polysaccharides are the dominant chemicals co-occurred in the decoctions. Small molecules are well-studied with multidisciplinary elaborations, whereas the role of polysaccharides remains largely elusive. As a case study, ginseng polysaccharides and ginsenosides in Du-Shen-Tang, the decoction of ginseng, were investigated on an over-fatigue and acute cold stress (OACS) model. UPLC-QTOF-MS/MS-based non-targeted metabolomics was employed to find gut microbiota related biomarkers. The diversity of gut microbiota before and after administration of ginseng polysaccharides was evaluated by 16S rRNA gene sequencing, while HPLC-TQ-MS/MS-based targeted metabolomics was used to investigate the pharmacokinetics and metabolisms of ginsenosides. The results indicated that ginseng polysaccharides improved the intestinal metabolism and absorption of certain ginsenosides (such as ginsenosides Rg1, Rd, and 20(S)-Rg3), and meanwhile reinstated the perturbed holistic gut microbiota (indicated by the biomarkers, such as trimethylamine-N-oxide, 4-hydroxyphenylacetate, hippurate, citrate, isocitrate, and 4-methyl-phenol) in OACS rat model, particularly, enhanced the growth of Lactobacillus spp. and Bacteroides spp., two major metabolic bacteria of ginsenosides (Zhou et al, 2016). All these results suggested that polysaccharides could affect the metabolism and absorption of small molecular co-existent in TCMs decoctions, which is mediated by reinstating the dysbiosis of gut microbiota. These findings shed new light on scientization and rationalization of the classic TCMs decoctions in human health care.

2.8 Revealing synergistic actions of TCMs with chemical drugs

TCMs are often clinically prescribed with chemical drugs to increase the efficiency or reduce toxicity of chemical drugs in China. Cyclophosphamide (CP) has been widely used to treat various cancers. However, CP is often restricted due to its seriously side effects, especially hepatotoxicity. Ginseng has been clinically used with CP, but it is unknown how ginseng reduces hepatotoxicity of CP. Metabolic analysis was used jointly with molecular biotechnology to investigate the hepatoprotective effects of ginseng on CP-induced hepatotoxicity and the potential mechanisms involved (Zhu et al, 2015). UPLC-QTOF-MS/MS-based metabolomics revealed that 33 endogenous metabolites of the serum from CP-treated rats changed in diversity. Nineteen endogenous biomarkers were reversed significantly when treated together with ginseng. The 19 biomarkers were deduced related to two main pathways, i.e., GSH metabolism and primary bile acids synthesis. Based on the results revealed by metabolomics, ginseng induced expression of GCLC, GCLM, GS, and GST, which associate with the disposition of GSH, and the expression of FXR, CYP7A1, NTCP, and MRP 3, which plays important roles in the synthesis and transport of bile acids, were confirmed respectively. Furthermore, NRF 2, one of regulatory elements on the expression of GCLC, GCLM, GS, GST, NTCP, and MRP3, was confirmed to be up-regulated when ginseng was co-administrated. All these results suggested that ginseng could alleviate CP-induced hepatotoxicity via modulating the disordered homeostasis of GSH and bile acid, which might be mediated by inducing the expression of NRF 2 in liver (Zhu et al, 2015). This finding does rationalize the combinative application of ginseng and CP in the cancer therapy.

3. Perspectives

Our limited experiences showed that LC-MS-based metabolomics is a superior strategy for TCMs researches concerning the holistic perspectives. However there are
several limitations in metabolomics study including LC-MS-based metabolomics approach, such as lack of a platform that detects all metabolites simultaneously, lack of metabolite annotations in search databases, and low statistical power for enrichment analyses of metabolic pathways. Despite these limitations, it is believed that with detection sensitivity improved, operation procedure standardized, upstream omics (genomics, proteomics, etc.) integrated with, more biomarkers identified and database established, as well as more metabolism pathways elucidated, metabolomics strategy would be a powerful tool for in depth understandings, and thus contributing to modernization and internationalization of TCMs.

**Conflict of interest statement**

The authors declare no conflict of interest.

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