Chapter 8
Metabolomics Applications in Herbal Medicine

Kati Hanhineva and Markku Pasanen

Abstract Metabolomics analytics focuses on the concomitant measuring of a vast array of small molecule metabolites in a biological sample. These approaches are heavily technique-driven, and have recently been increasingly utilized within the context of herbal medicine. Metabolomics analytics has been used to identify composition or origin of an herbal supplement, or to investigate clinical safety and efficacy responses in animals and humans following treatment with herbal products. Two main approaches – mass spectrometry preceded by either liquid or gas chromatographic separation, and nuclear magnetic resonance – are the most widely used techniques, and the development of instrumentation will be the driving force in terms of analytical accuracy when developing "end-points" or "biomarkers" for the clinical use of herbals or any other therapy.

Keywords Clinical trial • Drug interaction • Mass spectrometry • Metabolism • Nuclear magnetic resonance • Phytocomplex • Toxicity

Abbreviations

ADME	Absorption, distribution, metabolism, excretion
GC/TOF MS	Gas chromatography-time of flight mass spectrometry
HPLC	High performance liquid chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PLS-DA	Partial least squares discriminant analysis

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UPLC/Q-TOF MS	Ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry
TCM	Traditional Chinese medicines

Introduction

Metabolomics (metabonomics/metabolic profiling) is – together with transcriptomics and proteomics – an integral component of the systems biology concept (Nicholson et al. 2004; Baker 2011; Blow 2008; Patti et al. 2012). Metabolomics analyses focus on investigating the composition of low molecular weight biomolecules and correlating their patterns and concentrations with phenotypic observations, typically after various perturbations depending on the study focus (e.g., diet, medical/supplement treatment, stress, physical exercise, state of growth, or diseases). A metabolomics study setup involves the extraction of metabolites, analysis in a suitable platform (typically MS or NMR), data acquisition, collection, preprocessing, and statistical evaluation (Fig. 8.1). The metabolite alterations caused by the perturbations are revealed with statistical and chemometric evaluations, including unexpected changes, which are neglected in traditional hypothesis-driven targeted analyses. Such wide-scale approaches have been enabled by the improvements in analytical technologies within the past decade that provide for an extremely accurate, sensitive, and high-throughput approach to explore the metabolite content in virtually any biological material. This capacity can be utilized to measure thousands of analytes concomitantly from minimal sample material in non-targeted metabolite profiling setups. For these reasons, the non-targeted metabolite examination is particularly useful in addressing research questions involving myriad chemical species, such as in the case of herbal medicine.

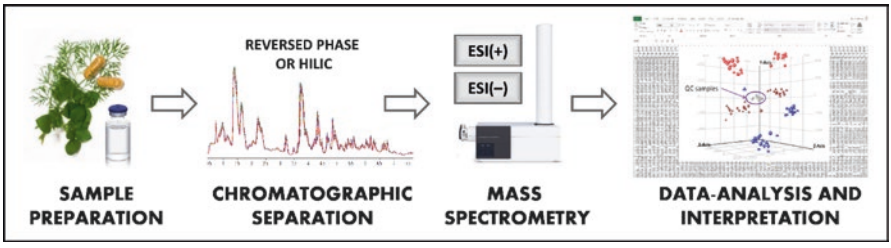

Fig. 8.1 Outline of the LC-MS based metabolomics analysis. The sample preparation typically involves straightforward one-step solvent extraction, followed by injection to the chromatographic separation and mass spectrometric analysis. The data are collected to large data matrices typically consisting of several thousand chemical entities, followed by pre-processing, statistical evaluation, metabolite identification, and biological interpretation utilizing various vendor-specific and open-source software

Herbal products or phytocomplexes used in herbal medicine typically contain complex mixtures of phytochemicals that can be characterized with large-scale metabolomics approaches more efficiently than by conventional targeted single-analyte methods. In particular, the non-targeted metabolite profiling approaches enable the examination of thousands of metabolic features on a single analytical experiment, and therefore offer a powerful tool for both the characterization of the phytochemical content in planta or in the used product, as well as in human borne samples when assessing the metabolic impact of the herbal products (Yuliana et al. 2013; AiHua et al. 2010; Zhang et al. 2012; Liu and Wang 2014). It offers a very wide window to observe the metabolite levels, which is inevitable for assessing the synergistic effects of the phytochemical mixtures on various cellular pathways. Metabolomics adopts a "top-down" strategy to reflect the function of organisms from terminal symptoms of metabolic network and understand metabolic changes of a complete system caused by various treatments in a holistic context.

Owing to its ability to systematically monitor vast metabolite content concomitantly, and thus provide a dynamic picture of the phenotype, one of the key areas where metabolomics is predicted to enable major achievements is drug discovery from traditional Chinese medicine. Traditional Chinese medicines (TCM) are a rich source of potential compounds for drug development, and the hypothesis-free metabolite profiling approach allows the analysis of a priori unknown chemicals, unlike targeted chemical analyses. Likewise, metabolomics allows for discovering biomarkers and perturbed pathways that can clarify the pharmacological mechanisms of traditional Chinese medicines, therefore offering the opportunity to scientifically express the meaning of evidence-based Chinese medicine (Zhang et al. 2012; Quan et al. 2014; Cao et al. 2015). Likewise, the possibility to utilize metabolite profiling approaches in the modernization of TCM preparations is foreseen, as the preparation of the traditional herbal products is complicated in practice. The optimization of modern extraction procedures may be done after more detailed knowledge about the traditional extracts' chemical profiles and their impact on biological activity is achieved with the aid of metabolite profiling, in order to obtain modernized extracts that contain the whole range of compounds relevant for the efficacy of the traditional application (Sheridan et al. 2012). Also, the possibility of using metabolomics analytics in the ADME evaluation of herbal products is foreseen, due to the ability of metabolomics approaches to capture a vast number of compounds and their potential products of metabolism in the evaluation of the pharmacokinetics and pharmacological mechanisms. The chemical diversity of compounds present in herbal products and the complex interactions they may have within cellular metabolism are not captured within traditional single-analyte biochemical measurements, and thus the capacity of metabolomics techniques is foreseen to aid in the analysis of mechanistic links between herbal-derived phytochemicals and cellular metabolic processes (Lan and Xie 2013; Xin et al. 2011; Wang and Chen 2013).

The two prevailing technologies utilized in metabolomics evaluation in general, as in herbal medicine, are mass spectrometry (MS) (Allwood and Goodacre 2010), and nuclear magnetic resonance (NMR) (Ludwig and Viant 2010; Schripsema

2010). While NMR has the advantage of providing actual quantities of analytes in focus and elucidating structures of unknown compounds, the major benefit from MS approaches is its superior sensitivity and therefore the ability to measure compounds in minimal, picomolar concentrations. Owing to this capacity to focus on low-level compounds, MS is typically more widely utilized in metabolomics experiments when addressing the phytochemical composition of plant-borne material, such as herbal supplements (Allwood and Goodacre 2010; Yang et al. 2012).

Metabolomics Approaches in the Characterization of the Phytochemical Content of Herbal Products

Until now, the area of herbal medicine where metabolomics approaches have been utilized most intensively by far is the characterization of the composition of bioactive phytochemicals in herbal plants and products made from them (Table 8.1). In particular, metabolite profiling has been proven useful in the quality assurance of herbal products. The authentication of herbal raw materials and products made from them is necessary in order to ensure the safety and efficacy of the products. The non-targeted metabolite profiling with large-scale data collection, followed by chemo-metric evaluation, has been proven to be a powerful tool for classifying even very closely related species that can otherwise be difficult to distinguish, based on morphological characteristics only. The metabolomics approach has been used as a classifier for seven *Lonicera* species flower buds, and the chemo-metric model created based on the metabolomics data showed good prediction performance (Gao et al. 2012). Likewise, the metabolite profile of curcuma extracts determined using gas chromatography-time of flight mass spectrometry (GC/TOF MS) and ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) enabled characterizing differences between *Curcuma aromatica* and *C. longa*

Table 8.1 Metabolomics applications in the analysis of herbal plants/products

Substance	Study focus	Method	Reference
Licorice species	Analysis of phytochemical composition	MS and NMR	Farag et al. (2012)
Echinacea species		MS	Hou et al. (2010)
Echinacea pallida (Nutt.)		MS	Pellati et al. (2012)
Lonicera	Quality assurance of herbal products	MS	Gao et al. (2012)
Asian and American ginseng		NMR	Zhao et al. (2015)
Panax ginseng and *Panax quinquefolius*		MS	Park et al. (2014)
Korean, Chinese and American ginseng		MS	Yang et al. (2013)
Curcuma	Identification of geographical origin of herbs	MS	Lee et al. (2014)
Schisandra chinensis		MS	Zhang et al. (2014)
Goji berries		MS	Bondia-Pons et al. (2014)
Angelica gigas		NMR and MS	Kim et al. (2011)

MS mass spectrometry, *NMR* nuclear magnetic resonance

grown in South Korea. The identified metabolites included several curcuminoids and terpenoids, and the metabolite profile allowed discriminating curcuma samples according to species or geographical origin (Lee et al. 2014). Also, the metabolite patterns of goji berries from various geographic origins were analyzed by LC-qTOF-MS metabolite profiling (Bondia-Pons et al. 2014). The different geographic origins clearly differentiate the replicative samples in principal component analysis, as shown in Fig. 8.2.

Ginseng is one of the most widely studied medicinal herbs, and publications describing its metabolomics applications are rapidly appearing. An NMR metabolomics approach was undertaken on 31 batches of ginseng from Chinese stores and assessed with a multi-step principal component analysis. Distinctive differences between Asian ginseng and American ginseng were found mainly in the levels of sucrose, glucose, arginine, choline, and 2-oxoglutarate and malate. Additionally, main differences between wild and cultivated ginseng were identified as ginsenosides (Zhao et al. 2015a). Also, the discrimination between *Panax ginseng* and *P. quinquefolius* has been proven feasible with the UPLC-QTOF-MS-based metabolic profiling method. This approach is very important since these two species have similar chemical and physical properties and the characteristics of their appearance are very similar, but the therapeutic effects are different (Park et al. 2014). Similarily, in another study, *Panax ginseng* Meyer (Korean origin and Chinese origin of Korean ginseng) and *P. quinquefolius* (American ginseng) were analyzed to investigate patterns in major metabolites using HPLC-based metabolic profiling. Partial least squares discriminant analysis (PLS-DA) showed a clear separation between *Panax*

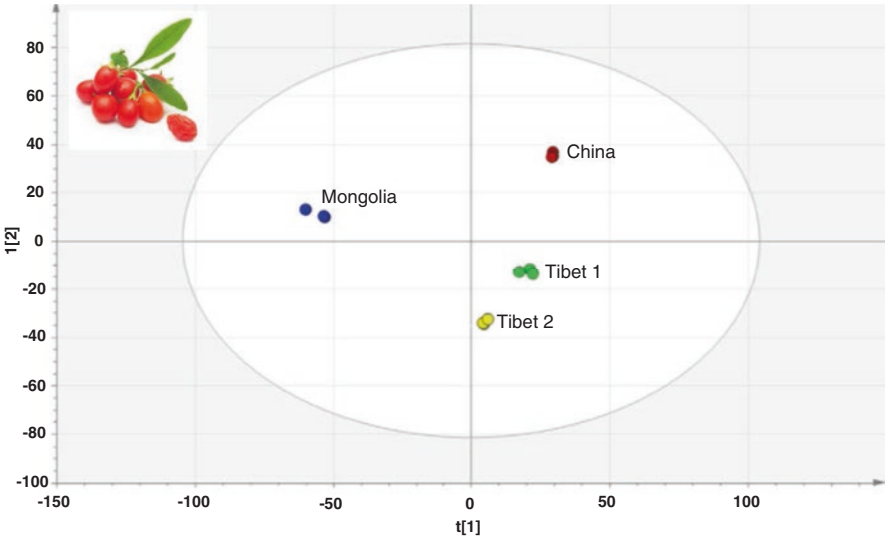

Fig. 8.2 Principal component analysis score plot derived from LC-qTOF-MS data using negative electrospray ionization of 12 Goji berry samples. Each circle represents a sample: Mongolian (*blue*), Chinese (*red*), Tibetan 1 (*green*), Tibetan 2 (*yellow*) (Reprinted from *Food Research International*, Volume 63: 132–138 Copyright 2014, with permission from Elsevier)

species and/or origins from different countries in the PLS-DA score plots, with various ginsenosides as the main differential compounds in the analysis (Yang et al. 2013). All the studies concluded that metabolite profiling can be used to undertake quality control of *Panax* products.

The geographical origin of Wu Wei Zi (*Schisandra chinensis*), an important herbal medicine mainly distributed in northeast China, was evaluated, based on a comprehensive metabolite profiling approach using GC-TOF-MS, ultra-performance LC (UPLC) quadrupole TOF (QTOF) MS. The different phytochemical composition of Wu Wei Zi from different areas including Heilongjiang, Liaoning, Jilin, and Shanxi of China was resolved with the robust and reliable method (Zhang et al. 2014). Furthermore, a combination of nuclear magnetic resonance (NMR) spectroscopy and ultraperformance liquid chromatography-mass spectrometry (UPLC-MS) followed by multivariate data analyses including principal component analysis and orthogonal partial least squares-discriminant analysis was used to characterize *Angelica gigas* obtained from other geographical regions in Korea (Kim et al. 2011).

In addition to the assessment of geographical origins or other quality control approaches, metabolite profiling techniques are widely used for the general, explorative characterization of the phytochemical composition of various herbal products. For example, various licorice species were characterized by large-scale metabolic profiling techniques to gain a broader insight into *Glycyrrhiza* species chemical composition, including *Glycyrrhiza glabra*, *G. uralensis*, *G. inflata* and *G. echinata*, which contained a plethora of phytochemicals, including terpenoids, saponins, flavonoids, polyamines, and polysaccharides (Farag et al. 2012).

Among the most widely used herbal supplements worldwide are various *Echinacea* preparations; however, the composition of different *Echinacea* plant species in the commercial *Echinacea* products is typically not well defined. A comparative metabolomics study by HPLC-MS was used to show that the three most used medicinal *Echinacea* species – *Echinacea purpurea*, *E. pallida*, and *E. angustifolia* – can be classified by the metabolite content, with alkamides and phenolic compounds as its main constituents (Hou et al. 2010). Another study focused on the detailed phytochemical characterization of *E. pallida* (Nutt.) root extracts and dietary supplements with a combination of HPLC with diode array and electrospray ionization-mass spectrometry (ESI-MS) detection (with ion trap and triple quadrupole mass analyzers). The quantitative analysis showed great variability in the number of the many bioactive compounds, including echinacoside and polyacetylenes and polyenes (tetradec-(8Z)-ene-11,13-diyn-2-one, pentedeca-(8Z,11Z)-dien-2-one and pentadec-(8Z)-en-2-one) (Pellati et al. 2012).

Metabolomics Investigations of In Vitro Studies in Herbal Medicine

Several studies, with numerous herbal medicine preparations or purified extracts of bioactive compounds from herbal products, have been published with various in vitro approaches using different animal or human-derived testing platforms,

Table 8.2 Metabolomics investigations of in vitro studies in herbal medicine

Substance	Study focus	Method	Reference
Echinacea	Role of alkylamides in the inhibition of CYP3A4	NMR and MS	Modarai et al. (2010)
Polygonum capitatum	Anti-tumor activity of ellagitannin fraction	MS	Ma et al. (2014)
Pyrrolizidine alkaloids	Metabolism of pyrrolizidine alkaloids	MS	Fashe et al. (2014), (2015a)
			Fashe et al. (2015a)

MS mass spectrometry, *NMR* nuclear magnetic resonance

including exposure of cell cultures (Table 8.2). Nowadays, increasingly, these studies use metabolite profiling techniques to gain a wider view on the metabolism of the herbal-derived chemicals, generation of metabolites, and cellular metabolism perturbations caused by the herb being studied.

Herbals have raised concern about drug-drug interactions in polypharmacy. One classic example is hyperforin fraction, which is isolated from St. John's Wort, which has proved to be a potent inhibitor of the human CYP3A4 enzyme and responsible for clinically significant interactions (Hohmann et al. 2015; Rahimi and Abdollahi 2012). Based on it, several approaches to identifying metabolites and subsequent interaction studies have been taken. For example, the metabolomic profiling of liquid *Echinacea* containing medicinal products has identified two alkylamides (dodeca-2 E,4 E,8 Z,10 E/Z-tetraenoic acid, and a new compound (putative molecular formula $C_{18}H_{36}NO(+)$), which are responsible for inhibiting CYP3A4 (Modarai 2010); however, its clinical significance is still unresolved. In another study using rat and human subcellular organelles and rat primary hepatocytes, altogether eight metabolites of ellagitannin polyphenols from *Polygonum capitatum* were successfully identified in the microsomes and isolated for further evaluations of pharmacologically active compounds (Ma et al. 2014).

Hepatotoxic and genotoxic pyrrolizidine alkaloids are often components and contaminants in herbal medicinal products and food or food supplements (EFSA CONTAM Panel 2011; EMA/HMPC/893108/2011 Committee on Herbal Medicinal Products 2014). Mimicking metabolomics platforms, a thorough metabolite profile evaluation in human subcellular organelles and LC/MS analysis introduced a new glutathione reactive metabolite, ((3H-pyrrolizin-7-yl) methanol) that can be formed from all pyrrolizidine alkaloids (Fashe et al. 2014, 2015a). Additionally, metabolite profiles between pyrrolizidine alkaloids sensitive and resistant species were different; more GSH-reactive metabolites were formed in sensitive species than resistant ones (Fashe et al. 2015b). On the other hand, although pyrrolizidine alkaloids are extensively metabolized and bioactivated by human CYP3A4 (Fashe et al. 2015a), based on the low quantity of parent compounds in any herbal or other source of exposure, they will not be responsible for any clinically relevant drug interactions, but toxic concerns still remain. Therefore, detailed in vitro approaches to identify generated metabolites and cellular responses using different test platforms of human origin will be of great value when establishing safety margins for herbal medicines.

Metabolomics Investigations of Herbal Medicine Studies Using Animal Experiments

The analysis of the biological/metabolic impact of herbal treatment often involves the use of animal experiments. Such analyses have superiority when compared to human trials in that they offer the possibility to examine the metabolic events perturbed with the herbal treatment also in various organs, rather than solely biofluid samples as is the case in human trials. In these experiments, the non-targeted metabolite profiling approaches are useful in providing detailed information about the herbal-derived compounds that potentially harbor the various organs, as well as monitoring the effect on the endogenous metabolite levels, which may aid in understanding the actual effect of the herbal treatment on a wider scale than possible when focusing on plasma (Table 8.3).

Metabonomic profiling in an experimental setting has been carried out, for example, in the examination of the chemopreventive effect of American ginseng (*Panax quinquefolius* L.). The serum metabolic alterations after treatment with ginseng suggested that the chemopreventive effects are exerted by anti-inflammatory and antioxidant mechanisms, as attenuation of impaired amino acids, carbohydrates, and lipid metabolism was observed in the GC-TOF-MS metabolomics analysis (Xie et al. 2015). An LC-qTOF-MS approach has been undertaken on a mouse model of systemic lupus erythematosus that was treated with jieduquyuziyin. The

Table 8.3 Metabolomics applications in the animal studies of herbal medicine

Substance	Study focus	Method	Reference
American ginseng	Chemoprevention in mouse	MS	Xie et al. (2015)
Jieduquyuziyin prescription	Effect on systemic lupus erythematosus in mouse	MS	Ding et al. (2014)
Chotosan	Effect on type 2 diabetes-induced dementia in mouse	MS	Niu et al. (2015)
Fu Fang Jin Jing	Effect on hypoxia and anxiety in mouse	NMR	Liu et al. (2013)
Da-Cheng-Qi	Effect on acute pancreatitis in rat	NMR	Li et al. (2015)
Chinese medicine including rhubarb, ethanol, and alpha- nephthylisothiolyanate	Pathogenic mechanism of yinhuang syndrome	MS	Tong et al. (2011)
Potentilla discolor	Treatment of type 2 diabetes in mouse	MS	Li et al. (2014)
Chaihuang-Yishen formula	Effect on diabetic nephropathy in rat	MS	Zhao et al. (2015)
Multi-component Chinese medicine (termed as SUB885C)	Effect on lipid biochemistry in mouse	MS	Weiet al. (2012)
Aristolochic acid	Induced nephrotoxicity in mouse	NMR	Tsai et al. (2013)

MS mass spectrometry, *NMR* nuclear magnetic resonance

orthogonal partial least squares analysis was used to analyze the metabolic patterns, and various compounds including, for example, phosphatidylethanolamine lipids and serotonin were linked in the pathogenesis of systemic lupus erythematosus. After treatment with jieduquyuziyin, the symptoms were alleviated, and metabolic differences were observed, following a suggestion that the herbal treatment could participate in the metabolism of unsaturated fatty acids, tryptophan, and phospholipids (Ding et al. 2014). Other animal studies with therapeutic approaches of various herbal medicines involving metabolomics include a focus on antidementia (Niu et al. 2015), anxiety (Liu et al. 2013), and acute pancreatitis (Li et al. 2015). An extreme approach to use metabolomics in an experimental setting was a study in which the pathogenic mechanism of Chinese medicine-induced yinhuang syndrome was examined in rats with a cocktail approach, and metabolomics data were collected on the organ level (Tong et al. 2011).

The potential role of herbal remedies in ameliorating or preventing type 2 diabetes has been widely investigated in various mouse models; these include the effect of *Potentilla discolor* in the treatment of type 2 diabetes mellitus in male C57BL/6 mice assessed with UPLC-Q-TOF-MS (Li et al. 2014), and the effect of Chaihuang-Yishen formula on rats with diabetic nephropathy by metabolomic and lipidomic analysis (Zhao et al. 2015b). A multi-component preparation used in Chinese medicine (termed as SUB885C) was administered to apolipoprotein E3 Leiden cholesteryl ester transfer protein (ApoE*3Leiden. CETP) mice followed by plasma and liver lipidomics analysis (Wei et al. 2012).

Metabolomics in Addressing the Effect of Herbal Treatment in Clinical Trials

The number of metabolomics analyses of controlled clinical trials with treatment by herbal medicines is still relatively low. Metabolomics approaches have been used when addressing the composition of herbal chemicals found in urine or plasma, and for evaluating various biomarker alterations related to endogenous metabolism in this context (Table 8.4). Patients'/subjects' responsiveness to herbal medicines and their efficacies have been followed by monitoring the patients'/subjects' endogenous metabolism including for example, carbohydrates, lipids, micronutrients, and vitamins in blood and/or urine samples. One goal of such research has been the identification of biomarker candidates to be used as an endpoint for pharmacodynamic responses. Clinical situations when such approaches have been used are, among others, depression (Tian et al. 2014), metabolic disorders including diabetes (van Wietmarschen et al. 2013), dysmennorrhea (Su et al. 2013), and hypertension (Feng et al. 2015). The duration of the study in the above-mentioned trials has varied between four and 12 weeks, and in some cases each subject acted as his or her own control during the trial. In the depression study (Tian et al. 2014), using the Hamilton depression scale scoring therapeutic cure, eight pharmacodynamic-like

Table 8.4 Metabolomics applications in human trials of herbal medicines

Substance	Study focus	Method	Reference
TCM formula Xiaoyaosan	Effect on depression	NMR	Tian et al. (2014)
Rehmannia six formula (R6)	Effect on clinical chemistry and metabolite profiles	MS	van Wietmarschen et al. (2013)
Shaofu Zhuyu formula concentrated-granule, SFZYFG	Effect on primary dysmenorrhea	MS	Su et al. (2013)
Qingrehuatan decoction	Effect on hypertension	NMR	Feng et al. (2015)

MS mass spectrometry, *NMR* nuclear magnetic resonance

metabolomics components increased or decreased as a response to the herbal therapy (urinary creatinine, taurine, 2-oxoglutarate and xanthurenic acid, citrate, lactate, alanine, and dimethylamine). The authors suggest that they can also be useful biomarkers for efficacy. However, in the metabolic disorders trial, clinical efficacy was mainly estimated according to the traditional Chinese medicine endpoints, but the relevant endpoints generally used in Western medicine (e.g., blood glucose, insulin, HbA1c, and triglycerides) were not used to establish clinical efficacy (van Wietmarschen et al. 2013). Metabolic endpoints were measured in serum where LDL-C, total cholesterol and phosphatidylcholine levels were decreased during the therapy. In the dysmenorrhea trial (Su et al. 2013), both plasma and urine were collected from patients as well as from healthy controls. Altogether, 19 metabolites in plasma and 16 metabolites in urine were up- or down-regulated, compared to the controls. These metabolic pathways represent sphingolipid metabolism, steroid hormone biosynthesis, and glycerophospholipid metabolism, and were considered to be pharmacodynamics efficacy signals. In the hypertension trial, serum samples were analyzed from 12 young hypertensive patients with phlegm-heat syndrome before and after four weeks on herbal therapy. Following the therapeutic response, altogether nine metabolite markers were increased, whereas five metabolites were decreased. (Feng et al. 2015).

One common feature for all the above-mentioned clinical examples is that the studies were not designed to cover present regulatory guidance (e.g., European Medicines Agency guidelines according to each therapeutic area). Rather, each study represents an experimental preliminary approach to identifying a "proof-of-concept" for the platform. However, based on these sporadic experiences, metabolomics approaches, together with indication-based true clinical hard endpoints, may finally serve patient monitoring, and after validation and qualification could establish clinical efficacy as "stand-alone."

Investigation of Adverse Effects of Herbal Medicines by Metabolomics

Herbal medicines are not always beneficial for the user. In the past, several cases were reported, in which the main reason for adverse, toxic, or even lethal responses were due to herbals (Teschke 2015). Based on these examples, in addition to other

"omics" techniques, metabolomics data could be supportive when analyzing the mechanism(s) of adverse responses to the drug. With this goal in mind, various compositions of aristolochic acid in mice were studied together with histopathological end points. The main outcome of the study was that despite the origin or composition of aristolochic acid, all material studied proved to be nephrotoxic in the proximal tubular area. This study reinforced the earlier data about toxic characteristics of aristolochic acid; it is toxic itself, and toxicity is dependent on the dosage of "active substance" only (Tsai et al. 2013). The above-mentioned example is not a gold standard for studies aimed at evaluating potential adverse responses by metabolomics means, but at least it shows that when several techniques are prospectively used to identify warning signals, the targets can be identified in more detail.

Conclusions

Metabolomics has been used, and will increasingly be used, to identify the source of material, to trace potential contaminants, and, finally, to identify efficacy and safety variables both in vitro and in vivo. These last two uses are still too premature to be fully taken into account as clinically relevant end-points as a response to herbal medicine, or to any other therapy. However, together with extensive, well-defined clinical trials with clinical end-points, metabolomic biomarkers could well serve regulatory purposes as well. What can be expected in future studies on herbal medicines? There is no easy answer, but by using modern analytical approaches and "omics" in in vitro platforms, certain hazards can be identified. On the other hand, prospective metabolomics approaches in clinical trials with difficult clinical end-points will most likely serve well for investigations on both safety and efficacy.

References

AiHua Z, Hui S, Wang ZG, WenJun S et al (2010) Metabolomics: towards understanding traditional Chinese medicine. Planta Med 76:2026–2035

Allwood JW, Goodacre R (2010) An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. (Special Issue: metabolomics in plant and herbal medicine research). Phytochem Anal 21:33–47, many ref

Baker M (2011) Metabolomics: from small molecules to big ideas. Nat Meth 8:117–121

Blow N (2008) Metabolomics: biochemistry's new look. Nature 455:697–700

Bondia-Pons I, Savolainen O, Törrönen R, Martinez JA et al (2014) Metabolic profiling of Goji berry extracts for discrimination of geographical origin by non-targeted liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Food Res Int 63:132–138, Part B

Cao H, Zhang A, Zhang H, Sun H, Wang X (2015) The application of metabolomics in traditional Chinese medicine opens up a dialogue between Chinese and Western medicine. Phytother Res 29:159–166

Ding X, Hu J, Wen C, Ding Z et al (2014) Rapid resolution liquid chromatography coupled with quadrupole time-of-flight mass spectrometry-based metabolomics approach to study the effects of jieduquyuziyin prescription on systemic lupus erythematosus. PLoS One 9:e88223

EFSA CONTAM Panel (2011) Scientific opinion on pyrrolizidine alkaloids in food and feed. EFSA J 9:2406–2540

EMA/HMPC/893108/2011 Committee on Herbal Medicinal Products. Public statement on the use of herbal medicinal products containing toxic, unsaturated pyrrolizidine alkaloids (PAs). 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2014/12/WC500179559.pdf

Farag MA, Porzel A, Wessjohann LA (2012) Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS, LC-MS and 1D NMR techniques. Phytochemistry 76:60–72, 45 ref

Fashe MM, Juvonen RO, Petsalo A, Rahnasto-Rilla M et al (2014) Identification of a new reactive metabolite of pyrrolizidine alkaloid retrorsine: (3H-pyrrolizin-7-yl) methanol. Chem Res Toxicol 27:1950–1957

Fashe MM, Juvonen RO, Petsalo A, Vepsalainen J et al (2015a) In silico prediction of the site of oxidation by cytochrome P450 3A4 that leads to the formation of the toxic metabolites of pyrrolizidine alkaloids. Chem Res Toxicol 28:702–710

Fashe MM, Juvonen RO, Petsalo A, Rasanen J, Pasanen M (2015b) Species-specific differences in the *in vitro* metabolism of Lasiocarpine. Chem Res Toxicol 28(10):2034–2044

Feng X, Yang Z, Chu Y, Du B et al (2015) 1H nuclear magnetic resonance-based metabolomic study on efficacy of Qingrehuatan decoction against abundant phlegm-heat syndrome in young adults with essential hypertension. J Tradit Chin Med 35:28–35

Gao W, Yang H, Qi LW, Liu EH et al (2012) Unbiased metabolite profiling by liquid chromatography-quadrupole time-of-flight mass spectrometry and multivariate data analysis for herbal authentication: classification of seven Lonicera species flower buds. J Chromatogr A 1245:109–116

Hohmann N, Maus A, Carls A, Haefeli WE, Mikus G (2015) St. John's wort treatment in women bears risks beyond pharmacokinetic drug interactions. Arch Toxicol 90(4):1013–1015

Hou CC, Chen CH, Yang NS, Chen YP et al (2010) Comparative metabolomics approach coupled with cell- and gene-based assays for species classification and anti-inflammatory bioactivity validation of Echinacea plants. J Nutr Biochem 21:1045–1059, 54 ref

Kim EJ, Kwon J, Park SH, Park C et al (2011) Metabolite profiling of Angelica gigas from different geographical origins using 1H NMR and UPLC-MS analyses. J Agric Food Chem 59:8806–8815

Lan K, Xie G, Jia W (2013) Towards polypharmacokinetics: pharmacokinetics of multicomponent drugs and herbal medicines using a metabolomics approach. Evid Based Complement Alternat Med 2013:819147

Lee J, Jung Y, Shin JH, Kim HK et al (2014) Secondary metabolite profiling of Curcuma species grown at different locations using GC/TOF and UPLC/Q-TOF MS. Molecules 19:9535–9551

Li Y, Li JJ, Wen XD, Pan R et al (2014) Metabonomic analysis of the therapeutic effect of Potentilla discolor in the treatment of type 2 diabetes mellitus. Mol Biosyst 10:2898–2906

Li J, Zhu SF, Zhao XL, Liu YX et al (2015) Metabolomic profiles illuminate the efficacy of Chinese herbal Da-Cheng-Qi decoction on acute pancreatitis in rats. Pancreatology 15:337–343

Liu P, Wang P (2014) Application of metabolomics technology in the research of Chinese medicine. Chin J Integr Med 20:307–310

Liu X, Zhu W, Guan S, Feng R et al (2013) Metabolomic analysis of anti-hypoxia and anti-anxiety effects of Fu Fang Jin Jing oral liquid. PLoS One 8:e78281

Ludwig C, Viant MR (2010) Two-dimensional J-resolved NMR spectroscopy: review of a key methodology in the metabolomics toolbox. (Special Issue: Metabolomics in plant and herbal medicine research.). Phytochem Anal 21:22–32, many ref

Ma JY, Zhou X, Fu J, Hu T et al (2014) Metabolite profiling analysis of FR429, an ellagitannin purified from Polygonum capitatum, in rat and human liver microsomes, cytosol and rat primary hepatocytes in vitro. Chem Biol Interact 220:33–40

Modarai M, Yang M, Suter A, Kortenkamp A, Heinrich M (2010) Metabolomic profiling of liquid Echinacea medicinal products with in vitro inhibitory effects on cytochrome P450 3A4 (CYP3A4). Planta Med 76:378–385

Nicholson JK, Holmes E, Lindon JC, Wilson ID (2004) The challenges of modeling mammalian biocomplexity. Nat Biotechnol 22:1268–1274

Niu Y, Li F, Inada C, Tanaka K et al (2015) Chemical profiling with HPLC-FTMS of exogenous and endogenous chemicals susceptible to the administration of chotosan in an animal model of type 2 diabetes-induced dementia. J Pharm Biomed Anal 104:21–30

Park HW, In G, Kim JH, Cho BG et al (2014) Metabolomic approach for discrimination of processed ginseng genus (Panax ginseng and Panax quinquefolius) using UPLC-QTOF MS. J Ginseng Res 38:59–65

Patti GJ, Yanes O, Siuzdak G (2012) Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 13:263–269

Pellati F, Orlandini G, Benvenuti S (2012) Simultaneous metabolite fingerprinting of hydrophilic and lipophilic compounds in Echinacea pallida by high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometry detection. J Chromatogr A 1242:43–58

Quan Y, Wang ZY, Xiong M, Xiao ZT, Zhang HY (2014) Dissecting traditional Chinese medicines by omics and bioinformatics. Nat Prod Commun 9:1391–1396

Rahimi R, Abdollahi M (2012) An update on the ability of St. John's wort to affect the metabolism of other drugs. Expert Opin Drug Metab Toxicol 8:691–708

Schripsema J (2010) Application of NMR in plant metabolomics: techniques, problems and prospects. (Special Issue: Metabolomics in plant and herbal medicine research). Phytochem Anal 21:14–21, many ref

Sheridan H, Krenn L, Jiang RW, Sutherland I et al (2012) The potential of metabolic fingerprinting as a tool for the modernisation of TCM preparations. (Special Issue: good practice in traditional Chinese medicine research in the post-genomic era). J Ethnopharmacol 140:482–491, many ref

Su S, Duan J, Wang P, Liu P et al (2013) Metabolomic study of biochemical changes in the plasma and urine of primary dysmenorrhea patients using UPLC-MS coupled with a pattern recognition approach. J Proteome Res 12:852–865

Teschke R, Eickhoff A (2015) Herbal hepatotoxicity in traditional and modern medicine: actual key issues and new encouraging steps. Front Pharmacol 6:72

Tian JS, Peng GJ, Gao XX, Zhou YZ et al (2014) Dynamic analysis of the endogenous metabolites in depressed patients treated with TCM formula Xiaoyaosan using urinary (1) H NMR-based metabolomics. J Ethnopharmacol 158(Pt A):1–10

Tong X, Sun H, Yan GL, Dong W et al (2011) Evaluation study on urine metabolomics in yinhuang rat model induced by triplet factors of rhubarb, ethanol, and alpha-nephthylisothiolyanate. Chin J Integr Med 17:369–375

Tsai DM, Kang JJ, Lee SS, Wang SY et al (2013) Metabolomic analysis of complex Chinese remedies: examples of induced nephrotoxicity in the mouse from a series of remedies containing aristolochic acid. Evid Based Comp Alternat Med 2013:263757

van Wietmarschen HA, van der Greef J, Schroen Y, Wang M (2013) Evaluation of symptom, clinical chemistry and metabolomics profiles during Rehmannia six formula (R6) treatment: an integrated and personalized data analysis approach. J Ethnopharmacol 150:851–859

Wang L, Chen C (2013) Emerging applications of metabolomics in studying chemopreventive phytochemicals. AAPS J 15:941–950

Wei H, Hu C, Wang M, van den Hoek AM et al (2012) Lipidomics reveals multiple pathway effects of a multi-components preparation on lipid biochemistry in ApoE*3Leiden. CETP mice. PLoS One 7:e30332

Xie G, Wang CZ, Yu C, Qiu Y et al (2015) Metabonomic profiling reveals cancer chemopreventive effects of American ginseng on colon carcinogenesis in Apc(Min/+) mice. J Proteome Res 14:3336–3347

Xin GZ, Qi LW, Shi ZQ, Li P et al (2011) Strategies for integral metabolism profile of multiple compounds in herbal medicines: pharmacokinetics, metabolites characterization and metabolic interactions. Curr Drug Metab 12:809–817

Yang M, Cheng C, Yang J, Guo DA (2012) Metabolite profiling and characterization for medicinal herbal remedies. Curr Drug Metab 13:535–557

Yang SO, Lee SW, Kim YO, Sohn SH et al (2013) HPLC-based metabolic profiling and quality control of leaves of different Panax species. J Ginseng Res 37:248–253

Yuliana ND, Jahangir M, Verpoorte R, Choi YH (2013) Metabolomics for the rapid dereplication of bioactive compounds from natural sources. Phytochem Rev 12:293–304, 54 ref

Zhang AH, Sun H, Wang X (2012) Recent highlights of metabolomics for traditional Chinese medicine. Pharmazie 67:667–675, many ref

Zhang Y, Li F, Huang F, Xie G et al (2014) Metabolomics analysis reveals variation in Schisandra chinensis cetabolites from different origins. J Sep Sci 37:731–737

Zhao H, Xu J, Ghebrezadik H, Hylands PJ (2015a) Metabolomic quality control of commercial Asian ginseng, and cultivated and wild American ginseng using (1) H NMR and multi-step PCA. J Pharm Biomed Anal 114:113–120

Zhao T, Zhang H, Zhang X, Zhao T et al (2015b) Metabolomic and lipidomic study of the protective effect of Chaihuang-Yishen formula on rats with diabetic nephropathy. J Ethnopharmacol 166:31–41