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Advanced applications of mass spectrometry imaging technology in quality control and safety assessments of traditional Chinese medicines

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Abstract

Ethnopharmacological relevance: Traditional Chinese medicines (TCMs) have made great contributions to the prevention and treatment of human diseases in China, and especially in cases of COVID-19. However, due to quality problems, the lack of standards, and the diversity of dosage forms, adverse reactions to TCMs often occur. Moreover, the composition of TCMs makes them extremely challenging to extract and isolate, complicating studies of toxicity mechanisms.

Aim of the review: The aim of this paper is therefore to summarize the advanced applications of mass spectrometry imaging (MSI) technology in the quality control, safety evaluations, and determination of toxicity mechanisms of TCMs.

Materials and methods: Relevant studies from the literature have been collected from scientific databases, such as PubMed, Scifinder, Elsevier, and Google Scholar using the keywords MSI, traditional Chinese medicines, quality control, metabolomics, and mechanism.

Results: MSI is a new analytical imaging technology that can detect and image the metabolic changes of multiple components of TCMs in plants and animals in a high throughput manner. Compared to other chemical analysis methods, such as liquid chromatography-mass spectrometry (LC-MS), this method does not require the complex extraction and separation of TCMs, and is fast, has high sensitivity, is label-free, and can be performed in high-throughput. Combined with chemometrics methods, MSI can be quickly and easily used for quality screening of TCMs. In addition, this technology can be used to further focus on potential biomarkers and explore the therapeutic/toxic mechanisms of TCMs.

Conclusions: As a new type of analysis method, MSI has unique advantages to metabolic analysis, quality control, and mechanisms of action explorations of TCMs, and contributes to the establishment of quality standards to explore the safety and toxicology of TCMs.

1. Introduction

Traditional Chinese medicines (TCMs) have been used in the clinic for thousands of years and have shown good therapeutic effects. Due to the complexity of components and the characteristics of multi-target actions, TCMs can be used for broad opportunities, but face severe challenges. Given their various types, qualities, and efficacies, the key to the modernization of TCMs is to study their material bases, discover their therapeutic or toxic components, control their qualities, and clarify their targets and mechanisms of action.

A variety of analytical methods have been used for the identification and mechanistic evaluation of individual TCM components, and can be mainly divided into two categories: chromatographic methods (including gas chromatography (GC) and hyphenated techniques (Zhang et al., 2013)), liquid chromatography (LC) and hyphenated techniques...
Chinese herbal medicines in tissues and cells, and perform rapid quality identification and locate the differential distribution of various metabolites in tissues, but may cause the loss of substances in low abundance (Prideaux and Stoeckli, 2012). FT-IR and NIR are spectroscopic methods that are non-invasive, rapid, and require simple sample preparations. However, their accuracies are lower than that of GC-MS and LC-MS. NMR has high resolution and signal quality to show the distribution of drugs and small molecule metabolites, but would destroy information on the distribution of compounds in tissues, without requiring special labeling or complex pre-treatment, which can not only identify and analyze substances but also reveal possible mechanisms of action of TCM components. This review aims to promote the application of MSI technology in Chinese herbal medicine and provide new directions for the discovery of drugs and the establishment of quality control standards for TCMs.

2. MSI: insights into the principles, indicators, and experimental processing

As a new type of molecular imaging technology, MSI performs mass spectrometry imaging and image visualization with high sensitivity, wide coverage, and strong identification ability. A variety of ions on the surface of tissue samples can be ionized point-by-point according to the spatial and multi-dimensional data of the mass to charge ratio (m/z), intensity, and position of ionized molecules obtained by mass spectrometry. Such data can be reconstructed and visualized using software (such as MassImager (He et al., 2018)) with the MSI functions of qualitative, quantitative, and positioning (Qin et al., 2018; Römpp and Spengler, 2013; Takahashi et al., 2015). Compared to LC-MS and immunohistochemistry (IHC), MSI can perform high-throughput detection of substances (endogenous and exogenous metabolites) in tissue sections, without requiring special labeling or complex pre-treatment, which can not only identify and analyze substances but also reveal their spatial distributions and relative contents in tissues (Schwamborn and Caprioli, 2010).

MSI was originally developed based on matrix-assisted laser desorption/ionization (MALDI). Therefore, MALDI-MSI is the most.
widely used mass spectrometry imaging method (Caprioli et al., 1997). In addition, related technologies include desorption electrospray ionization-mass spectrometry imaging (DESI-MSI), secondary ion mass spectrometry (SIMS) imaging, and laser ablation-inductively coupled plasma-mass spectrometry imaging (LA-ICP-MSI), etc. (de Souza et al., 2020; Oppenheimer and Drexl, 2011; Parrot et al., 2018). These technologies are mainly classified according to their ionization mode: SIMS imaging uses a primary ion beam to bombard the surface of the sample, and then introduces secondary ions sputtered from the surface into the mass spectrometer for mass separation and determination (Yoon and Lee, 2018). MALDI-MSI mainly makes use of a matrix to absorb the laser energy and then transfers energy to sample molecules for ionization (Knochenmuss, 2006). DESI-MSI uses atomized charged droplets to hit the surface of the sample. After being hit by high-speed droplets, the sample is sputtered and then subjected to the mass spectrometer (Ila et al., 2007; Takáts et al., 2004). The ion source type, spatial resolution, sample preparation requirements, and other information of these three mass spectrometry imaging technologies are summarized in Table 1.

2.1. Critical indicators

Speed, spatial resolution, and sensitivity are critical indicators of MSI (Vestal et al., 2020). Speed is the main factor affecting the experimental time, and the scanning rate mainly depends on the influence of the laser frequency, mobile platform speed, and signal acquisition. The increase in scanning speed leads to a decrease in ionized ions (Tillner et al., 2017). In this case, high sensitivity is key to ensuring the imaging results of low abundance ions. Spatial resolution and sensitivity are negatively correlated and an improvement in sensitivity will inevitably lead to a decrease in the mass resolution (Vestal et al., 2020). Sensitivity is also closely related to ionization efficiency, ion transport efficiency, and ion detection (Merdas et al., 2021), while the mass resolution is mainly dependent on the specific desorption/ionization method used (Handberg et al., 2015; Römp and Spengler, 2013) (Table 1). Therefore, MSI is a systematic project, in which the limit value of indicators should be selected according to the experiment.

2.2. Experimental process

We will use the most widely used technology, MALDI-MSI, as an example to describe the specific experimental process. First, the appropriate sample preparation method is selected according to the nature of the animal/plant tissue sample; a suitable matrix is selected for spraying based on the type and nature of the test object; a laser beam is used to desorb and ionize each sampling point. Subsequently, the analyte ions are separated and detected by the mass spectrometer to obtain the mass spectra associated with the sample space position. Finally, the MSI map is obtained by matching and reorganizing all of the mass spectra associated with the sample space position. Therefore, MSI is a systematic project, in which the limit value of indicators should be selected according to the experiment.

2.2.2. Matrix selection

In MALDI-MS analysis, the image quality depends in large part on the establishment and optimization of the matrix system, and thus, the choice and spray type for the matrix is very important. Commonly used matrices include sinapic acid (SA) (Chaurand et al., 2008), α-cyano-4-hydroxycinnamic acid (CHCA) (Grassl et al., 2011; Lemaire et al., 2006), 2-mercaptobenzothiazole (2-MBT) (Astigarraga et al., 2008), 2,5-dihydroxybenzoic acid (DHB) (Li et al., 2016b), 2,5-dihydroxyacetophenone (DHP) (Jovanović and Peter-Katalinić, 2016), 9-aminoacridine (9-AA) (Morikawa-Ichinose et al., 2019), and 1,5-diaminonaphthalene (DAN) (Korte and Lee, 2014). Among them, SA and DHB are suitable for the detection of high molecular weight biomolecules (proteins, oligosaccharides, etc.), CHCA and 2-MBT are fit for the detection of medium molecular weight analytes (peptides, lipids), and DHB, DAN, and 9-AA are preferred for the detection of low molecular weight molecules (fatty acids, amino acids, nucleotides, etc.). In addition, some novel matrices such as quercetin (Wang et al., 2014), N-phenyl-2-naphthylamine (Liu, H. et al., 2018), graphene oxide (Wang et al., 2017), 3,4-dimethoxyxycinnamic acid (DMCA) (He, H. et al., 2019), and poly-L-lysine (PLL) (He, Y. et al., 2019) have been successfully used for MALDI-MSI. After selecting the suitable matrix according to the sample type, it is necessary to evenly cover the matrix solution on the surface of the tissue section to form good co-crystallization with the tissue surface molecules. There are three main methods of matrix covering, including manual spraying, automatic spraying, and vacuum sublimation (Bjarnholt et al., 2014). Furthermore, matrix coating assisted by an electric field (MCAEF) has also been proven to enhance tissue imaging (Wang et al., 2015).

2.2.3. Data processing

MSI will obtain large volumes of mass spectral data during high-throughput detection, which can be reconstructed and visualized into image information using imaging software (such as MassImager (He et al., 2018), R Packages (Rölfs et al., 2020), MSIReader (Desbenoit et al., 2020), etc.)

Table 1

Comparison of the three most commonly used MSI techniques.

| Ionization type | Ionization source | Environment | Resolution | Characteristic | Ref. |
|----------------|-------------------|-------------|------------|---------------|-----|
| MALDI          | IR/UV             | High vacuum/low vacuum | IR : 150 μm, UV: 10–250 μm | Need matrix, wide detection range | Heyman and Dubery (2016) |
| SIMS           | Primary ion beam  | High vacuum | 50 nm–5 μm | High resolution, high vacuum, easy to produce fragments of ions | Behrens et al. (2012) |
| DESI           | Charged corpuscle | Atmospheric pressure | 100–200 μm | No matrix, atmospheric pressure | Ila et al. (2007); Takáts et al. (2004); Wiseman et al. (2006) |
generally required for LC-MS, including solvent extraction and chromatographic column separation before structural characterization. Such methods, particularly solvent extraction, are commonly used in the fields of medicine (Schulz et al., 2019; Vanderspek et al., 2015), food (Morisasa et al., 2019), and plant biology (Kasper et al., 2011; Korte et al., 2015; Qin et al., 2018). The MSI methods, research drugs, tissue types, and imaged molecules involved in this article are summarized in their order of appearance in Table 2.

3. MSI: A camera for showing the distribution of multiple components in a plant

Investigations of the basal metabolism of TCMs are the premise for identifying new drug candidates, increasing the clinical range of drugs, and improving quality control. Secondary metabolites (such as flavonoids, mushrooms, alkaloids, etc.) are the main components of TCMs that can prevent or cure diseases. The types, contents, and relative proportions of secondary metabolites are key to determining the effectiveness and quality of TCMs (Zhang et al., 2018) and MSI is suitable for detecting the content and distribution of primary/secondary metabolites in various plant structures (petals, roots, stems, leaves, seeds, seedlings) (Enomoto, 2020; Enomoto and Nirasawa, 2020; Qin et al., 2018; Sagara and Pradeep, 2013).

The conventional mass spectrometry method used to study the multiple components of TCMs is LC-MS. Complex pretreatment is generally required for LC-MS, including solvent extraction and chromatographic column separation before structural characterization. Such work not only requires substantial investigator energy and wastes a considerable amount of chemical reagents for sample preparation, but may also cause the loss of analytes or damage to the active ingredient (Wu et al., 2007). Furthermore, LC-MS fails to provide location information for the analyte in the tissue. Conversely, MSI can directly analyze the solid sections of plant tissues, without labeling and pre-processing and many studies have confirmed the advantages of direct analysis of plant tissues (Talaty et al., 2005; Wu et al., 2007). MSI can detect and identify the metabolic distribution of various components of TCMs while retaining in situ information, which is especially suitable for showing the material differences among different tissue parts of TCMs and the distribution characteristics of multiple components in the tissue (Hemalatha and Pradeep, 2013).

In studies of *Salvia miltiorrhiza*, MALDI-MSI was used to visualize the spatial dynamics of functional metabolites (such as amino acids, phenolic acids, fatty acids, oligosaccharides, cholines, etc.) (Sun et al., 2018) and MALDI-MSI was used to determine the distribution of metabolites in the tissue structures of roots, stems, and leaves. In this study, the characteristic constituents of the medicinal plant *Salvia miltiorrhiza* were identified as phenolic acids and tanshinones, which was consistent with the LC-MS data (Li et al., 2020b). MALDI-MSI was also used to identify and show the location of specific metabolites in *Tripterygium* roots (Lange et al., 2017). In a study of *Paonia lactiflora*, atmospheric pressure-scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI, 10 μm/30 μm resolution) was used to detail the specific distribution of the major secondary metabolites, gallotannins and monoterpenic glucosides, in root samples (Li et al., 2016a). SIMS imaging was used to characterize the morphological distribution of syringyl and guaiacyl lignin in the xylem of maple samples, which revealed a clear difference in the annual distribution of lignins between the fiber and vessel (Saito et al., 2012). Take *Pueraria lobata* as an example to illustrate in detail, the maytansinoids of *Pueraria lobata* were visualized by AP-SMALDI MSI in the rhizome and were highly distributed in the vascular cambium region and the phloem. Such compounds were also widely distributed in the xylem and extremely low in the outer bark (periderm) of the stem. In addition, maytamine and maytanic acid were mainly detected in the central cylinder of the root (Fig. 2)(Eckelmann et al., 2016).

Due to high background noise in the low mass (<500 Da) region and the spatial inhomogeneity of matrix crystals formed on plant tissues, the application of MSI to the analysis of small molecule metabolites in plant tissues is more challenging than that in animal tissues. To improve the spatial resolution of MSI, some new ion sources were constructed for plant tissue imaging. Plasma assisted laser desorption ionization mass
Table 2
Published literature showing the application of MSI for the composition, quality control, and mechanisms of action of TCMs and natural products.

| Drug                          | Tissue type | Technical method | Imaged molecules | Ref.       |
|-------------------------------|-------------|------------------|------------------|------------|
| Salvia miltiorrhiza           | Whole plant | MALDI-MSI        | Functional metabolites | Sun et al. (2020) |
| Salvia miltiorrhiza           | Roots, stems and leaves | MALDI-MSI | Phenolic acids and tanninones | Li et al. (2020b) |
| Tripterygium                  | Roots       | MALDI-MSI        | Triterpenoids and sesquiterpenoids | Lange et al. (2017) |
| Paonia lactiflora            | Roots       | AP-SMALDI MSI    | Galactolipids and monoterpenes | Li et al. (2016a) |
| Maple                        | Xylem       | TOF-SIMS imaging | Guaiacyl lignin | Saito et al. (2012) |
| Pouterickia pyracantha       | Stems and roots | MALDI-MSI | Maytansinoids | Eckelmann et al. (2016) |
| Scutellaria                    | Roots       | PALDI-based MSI  | Baicalin and wogonin | Feng et al. (2014) |
| Asclepias curassavica         | Injury site | MALDI-MSI        | Cardiac precursors | Dreisbach et al. (2021) |
| Glycyrrhiza glabra            | Rhirome     | AP-MSI           | Flavonoids, flavonoid glycosides and saponins | Wizemann et al. (2014) |
| Ginkgo biloba L.              | Leaves      | AP-MSI           | Flavonoids and biflavonoids | Beck and Stengel (2016) |
| Catharanthus roseus           | Stem tissue | MALDI-MSI        | TIA's | Yamamoto et al. (2016) |
| Catharanthus roseus           | Leaves      | MALDI-MSI        | TIA precursors and precursors | Yamamoto et al. (2019) |
| Panax ginseng                 | Roots       | MALDI-MSI        | Ginsenosides | Bai et al. (2016) |
| Ginseng                       | Roots       | DESI-MSI         | Gensenosides | Yang et al. (2021) |
| Panax ginseng, Panax quinquefolius, and Panax notoginseng | Roots | MALDI-MSI | Saponins | Wang et al. (2016) |
| Aconitum carmichaeli Deba     | Roots       | MALDI-MSI        | Aconitum alkaloids | Wang et al. (2009) |
| Paonia suffruticosa and Paonia lactiflora | Roots | MALDI-MSI | Monoterpenoids and paeonol glycosides, tannins, flavonoids, sascharides and lipids | Li et al. (2021) |
| Ligustri Lucidi Fructus (LLF) | LLF fruits  | MALDI-MSI        | Q-markers | Li et al. (2020a) |
| Vinblastine                   | The whole body of cats | MALDI-IMS-MSI | Sinblastine and metabolites | Trim et al. (2008) |
| Salidroside                   | Multiple organs | MALDI-MSI        | Salidroside | Meng et al. (2020) |
| Puerarin                      | Mice kidney tissue | GD-4 assisted MSI | Puerarin and its two metabolites (daidzein and dihydroredaiztein) | Shi et al. (2017) |
| Scutellaria                   | MALDI-MSI   | Scutellarin and scutellarin | Wang et al. (2021c) |

Table 2 (continued)

| Drug                          | Tissue type | Technical method | Imaged molecules | Ref.       |
|-------------------------------|-------------|------------------|------------------|------------|
| Notoginseng leaf              | Rat brain   | MALDI-MSI        | Endogenous metabolites | Wang et al. (2021b) |
| Notoginsenoside               | Rat brain   | MALDI-MSI        | Endogenous metabolites | Zhu et al. (2020) |
| Thymoquinione                 | Rat brain   | MALDI-MSI        | Endogenous metabolites | Tian et al. (2020) |
| Radix Aconitii Lateralis      | Rat heart   | MALDI-MSI        | Endogenous metabolites | Wu et al. (2019) |

spectrometry (PALDI-MSI) combines multiwavelength laser desorption and heated metastable plasma ionization of analytes, and does not require solvents to decrease ion suppression, reduce the pH effect, or simplify complicated spectra caused by adducts to a high spatial resolution of 60 μm × 60 μm. PALDI-based MSI for tissue section imaging of *Scutellaria baicalensis* showed that the two active components, baicalein, and wogonin, were mainly distributed in the epidermis of the root (Osakabe et al., 2014). To solve the problem of uneven matrix distribution in MALDI-MSI, colloidal graphite was introduced as an alternative matrix that can be evenly distributed on the sample surface. Colloidal graphite-assisted laser desorption/ionization (GALDI) MS imaging was developed to analyze the metabolites of *Arabidopsis*, showing the specific distribution of flavonoids in *Arabidopsis* in the whole flower and a single petal (Cha et al., 2008). In addition, 3D-MSI has been developed as cutting-edge technology for plant imaging. The 3D-surface MALDI-MSI is the most recent instrumental approach in AP-MALDI MSI and was developed to characterize the specific distribution of plant defensive cardiac glycosides at injury sites in *Asclepias curassavica* (Dreisbach et al., 2021).

Most of the MALDI imaging experiments performed on plant tissues have a spatial resolution of 50–200 μm. With high resolutions in mass and space, this technology has been applied to cell-level imaging in plants. The AP-MALDI-MSI approach (Koestler et al., 2008) that was independently developed by Li’s laboratory achieves 10 μm resolution in cell level imaging in plants, thus, showing the distribution of the main natural products (flavonoids, flavonoid glycosides, and saponins) of *Glycyrrhiza glabra* (licorice)(Li et al., 2014). The technology was also used to detect and identify the distribution of flavonoid glycosides and biflavonoids in *Ginkgo biloba* L. (Beck and Stengel, 2016). A study used MALDI-MSI based on the FT-ICR-MS detector (with a spatial resolution of 20 μm) to show that most of the terpenoid indole alkaloids (TIAs) in the stem tissue of *Catharanthus roseus* were accumulated in idioblast cells (ICs) and laticifer cells (LCs) (Yamamoto et al., 2016). Another study also used the FT-ICR-MS detector to image the leaves of *Catharanthus roseus* at a resolution of 10 μm, and was combined with single cell MS analysis to detail the biosynthesis of TIAs and determine the cell-specific localization of TIAs in leaf tissue (Yamamoto et al., 2019).

MSI technology can achieve high resolution cell and tissue imaging, showing the specific distribution of the functional metabolites of TCMs and laying a foundation for subsequent mechanistic exploration.

4. MSI: A simple and quick way to discover the quality markers of TCMs

Due to the polymorphism of medicinal plants, the quality control of drugs is a complicated process and includes a detailed characterization of the appearance, active ingredients, and physical and chemical properties of TCMs, as well as the quantification (absolute dry weight, yield, etc.), manufacturing (temperature, solvent, extraction and drying time),
impurity testing, and chemical content determinations of the final active pharmaceutical ingredients (Liu, C. et al., 2018). In recent years, to improve the consistency and quality control of TCMs, quality markers (Q-makers) have been introduced (Guo, 2017; Liu et al., 2016). Q-markers of TCMs refer to substances that can be characterized qualitatively and quantitatively and are closely related to the function of the pharmaceutical ingredients (Liu, C. et al., 2018). In recent years, to improve the consistency and quality control of TCMs, quality markers (Q-makers) have been introduced (Guo, 2017; Liu et al., 2016). Q-markers of TCMs refer to substances that can be characterized qualitatively and quantitatively and are closely related to the function of the pharmaceutical ingredients (Liu, C. et al., 2018). In recent years, to improve the consistency and quality control of TCMs, quality markers (Q-makers) have been introduced (Guo, 2017; Liu et al., 2016). Q-markers of TCMs refer to substances that can be characterized qualitatively and quantitatively and are closely related to the function of the pharmaceutical ingredients (Liu, C. et al., 2018).

As mentioned, MSI can detect the content and distribution of multiple components of TCMs in a high throughput manner. As a new analytical method, this technique has been used to discover the quality markers of TCMs. In this application, massive volumes of mass spectral data are generated and subsequently analyzed and processed by chemometric methods. Such methods mainly include principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), linear discriminate analysis (LDA), local least square (LLS), heuristic evolving latent projections (HELP), and orthogonal projection analysis (OPA) (Bansal et al., 2014). Compared to other chemical analyses such as LC-MS and UV, MSI does not require complicated sample extraction and separation steps, and does not lose low-abundance components. Thus, MSI quickly distinguishes the active ingredients and metabolic characteristics of different drugs, as well as readily identifies Q-markers. All such capabilities are suitable to rapidly and semi-quantitatively perform quality screening of TCMs (Huang et al., 2016).

Panax ginseng is a type of precious Chinese medicine, known as the king of medicines. However, as there are multiple species of Panax ginseng, the origin, age, efficacy, and nutritional value of ginseng medicines are also different, and counterfeit or substandard products often exist in the market. Ginsenosides are the main active component in Panax ginseng, and the content of ginsenoside increases with plant age. Many studies have used MSI to reveal that ginsenosides are mainly distributed in the sebaceous layer and part of the cortex of Panax ginseng tissue located in the center of the root. Dozens of ginsenoside analytes have been identified by MS/MS as specific markers for quickly distinguishing different varieties, ages, and organs of Panax ginseng based on their specific distributions in tissues (Fig. 3) (Bai et al., 2016; Lee et al., 2017; Taira et al., 2010; Wang et al., 2016; Yang et al., 2021). In one study, UPLC-QTOF MS and DESI-MSI were simultaneously used to detect and characterize the age and parts of ginseng to identify the common biomarkers across different age groups using the OPLS-DA method. The results showed that compared to UPLC-QTOF MS, DESI-MSI was a novel and stable method for the rapid evaluation of ginseng root slices (Yang et al., 2021). In addition, LC-MS and MALDI-MSI were also used to analyze Aconitum alkaloids in the Chinese herbal medicine, Aconitum carmichaeli Debx. The results between the two analytical methods were consistent and revealed significant differences in the contents of alkaloids between different samples. The comparative study using two analytical methods showed that MALDI-MSI was a more rapid and robust analytical method than LC-MS for semi-quantitative analyses of high concentration alkaloids (Wang et al., 2009). In addition, spatial metabolomics based on MALDI-MSI was also used to comprehensively and accurately detect the differential distribution of metabolites in Paeonia suffruticosa and Paeonia lactiflora (both belonging to genus Paeonia), including monoterpenes and paeonol glycosides, tannins, flavonoids, carbohydrates, and lipids, and it was also used to further visualize the gallotannins biosynthesis pathway in the roots of Paeonia suffruticosa and Paeonia lactiflora (Li et al., 2021). Most TCMs are crude drugs and the majority of which must be processed to reduce their...
Overlay of ion images: red, drug of interest; yellow, metabolites; green, background noise and ultra-high sensitivity. GD-4-assisted MSI has been used to show the distribution characteristics of puerarin and its metabolites in renal microregions showing that puerarin was primarily distributed in the renal pelvis and major calyx, whereas its metabolites (daidzein and dihydrodaidzein) were also detected in the renal pelvis, distributed in the renal pelvis and major calyx. However, its metabolites were nearly absent in the minor calyx, and partly in the major calyx, but were nearly absent in the medulla (Shi et al., 2017). In another study, MALDI-MSI was also used to visualize the temporal and spatial distribution of salidroside showing that salidroside was heterogeneously distributed throughout the kidney (Meng et al., 2018). By collecting multiple organ samples from mice at various time points after intravenous administration of salidroside, MALDI-MSI was used to discover Q-makers, providing a new direction and insights for the quality control of TCMs. As a rapid evaluation method, MSI has a broad applicability for the quality control of TCMs.

5. MSI: A tool for studying the metabolic distribution and therapeutic/toxic mechanisms of TCMs

MSI can be applied to entire animal bodies or multiple tissue sections to observe the distribution of metabolites of active components in each organ, and to determine the target organ and toxicity.

A study on the anticancer drug, vinblastine, performed MALDI-MSI to visualize the distribution of its metabolites in the liver, renal cortex, and heart, and could be quickly eliminated with 5 min (Meng et al., 2020). The distribution of drugs in tumor tissues or organs is heterogeneous. The possible metabolic pathways of TCMs can also be predicted by MSI to analyze the distribution of active components and their metabolites in microregions of tissues or organs. It has been found that hydroxyl-group-dominated graphite dots (GD) are an ideal matrix with extremely low background noise and ultra-high sensitivity. GD-4-assisted MSI has been used to show the distribution characteristics of puerarin and its metabolites in renal microregions showing that puerarin was primarily distributed in the renal pelvis and major calyx. However, its metabolites (daidzein and dihydrodaidzein) were also detected in the renal pelvis, major calyx, and partly in the minor calyx, but were nearly absent in the medulla (Shi et al., 2017). In another study, MALDI-MSI was also used to identify the in situ localization of scutellarin (traditional Chinese botanical drug of Erigeron brevisscapus extract) and its metabolites to show metabolic differences in the kidney (Wang et al., 2021d). Imaging the distribution characteristics after drug administration facilitates an understanding of the biological activity and metabolism of drugs in various animal organs.

In recent years, various cutting-edge omics technologies (genomics, transcriptomics, proteomics, metabolomics, lipidomics) have been applied to diverse fields of TCM research, including screening, quality control, research and development, mechanistic research, and clinical verification. Taking metabolomics as an example, metabolism reflects the changes of small molecule metabolites in the body. Metabolomics with high-throughput monitoring can identify the metabolic network of molecules following drug administration, which has become a powerful tool effectively breaking through the application bottleneck of the study of the multi-component mechanisms of TCMs. The discovery of metabolomic markers provides a foundation for the early identification of toxicity, quality control, and clinical utility of TCMs (Han et al., 2020; Shi et al., 2016; Sun et al., 2012; Wang et al., 2021a). As MSI is a high-throughput and label-free technology, it can obtain drug metabolism distribution information and also endogenous small molecule metabolism information (that is, metabonomics data) from the same animal tissue. Compared to traditional metabolomics methods, spatial high-resolution metabolomics studies based on MSI can preserve tissue integrity and visualize the distribution of metabolites. Researchers can also superimpose MS images with optical/HE scanning images and focus on the tissue microregions or lesions of interest to accurately extract mass spectral data for the target area for metabolic research; thus, avoiding the challenges associate with difficult separations of research specimens.

Panax notoginseng is a traditional Chinese medicine and is widely used for the treatment and prevention of ischemic cerebrovascular diseases (Yan et al., 2018). Notoginseng leaf triterpenes (PNGL) and notoginsenoside R1 (NG-R1, Fig. 4) extracted from Panax notoginseng were visualized by MALDI-MSI to study the effect on small molecule metabolism after perfusion injury. According to the results, the two drugs had a callback effect on the tricarboxylic acid (TCA) cycle and adenosine triphosphate (ATP) metabolism pathway, and also played a role in improving the malate-aspartate shuttle; thus, improving the antioxidant capacity and maintaining the homeostasis of Na⁺ and K⁺ (Wang et al., 2021b; Zhu et al., 2020). In the similar disease model, Fang et al. also used MALDI-MSI to explore the role of Thymoquinone, the main active ingredient in Nigella sativa, in regulating abnormal metabolism in injured brain areas by promoting the aerobic oxidation of glucose, regulating intracellular energy metabolism, improving the...
phospholipid molecular level, increasing the content of small antioxidant molecules, and balancing sodium homeostasis (Tian et al., 2020). According to the results of metabolomics studies in the same model, it is known that the mechanisms of related drugs for the treatment of stroke and other central nervous system diseases begin with mitochondrial oxidative damage, energy metabolism, lipid metabolism disorders, and Na\(^+\) homeostasis. In another study, MALDI-MSI was used to study anti-myocardial infarction effects of Radix Aconiti Lateralis Preparata extracts. Pharmacodynamics results showed that Radix Aconiti Lateralis Preparata extracts can improve the hemodynamic status and organ weight index and inhibit myocardial injury of rats with myocardial infarction. The corresponding MALDI-MSI results elucidated the possible mechanism of action by presenting Radix Aconiti Lateralis Preparata extracts to reverse metabolic changes of related small molecules (energy metabolism-related molecules, phospholipids, potassium ions, and glutamine in the heart) to produce anti-myocardial infarction effects (Wu et al., 2019). The identification of potential biomarkers of TCMs based on changes in the metabolic networks of small molecules in vivo, thus, lays a foundation for further exploration of the mechanisms of action.

Spatial metabolomics based on MSI can detail the interactions between metabolites, and further screen and identify biomarkers with significant changes by comparing the correlation between metabolomics spectra and histopathological/biochemical indicators. Finally, the analysis of related metabolic pathways can reveal the possible effects or toxic mechanisms of TCMs. The above studies illustrate that spatial metabolomics analyses based on MSI methods are powerful in exploring the therapeutic effects of TCMs and provide insights into the potential mechanisms of action of TCMs.

6. Summary and conclusion

In recent years, MSI has attracted the attention of many researchers and was rapidly developed. Currently, the quality control of most TCMs is limited to the identification and analysis following extraction and separation, and the process is cumbersome and time-consuming. The ingredients with lower concentrations are often overlooked and are not the focus of studies. MSI provides a new method for the rapid screening and control of the quality of TCMs. The understanding of modern medicine in TCMs has developed from macroscopic to microscopic considerations. In particular, the discovery and identification of active components of TCMs in the body is a key research topic. MSI technology has become a powerful tool for the analysis of metabolites in animal/plant tissues, as well as single cells, providing a means to study transport pathways, metabolic pathways, and the accumulation of exogenous drugs in animal tissues and endogenous metabolites in plant tissues. The multi-component and multi-target synergistic characteristics of TCMs have been advantageous for the treatment of certain chronic diseases. Extracting active ingredients from TCMs and isolating monomers is a key approach to the identification of new drugs. MSI also offers a new visual perspective and provides multi-dimensional information for metabolomics analysis. However, MSI technology has faced many challenges, such as its limited spatial resolution and insufficient sensitivity. By improving sample preparation methods, matrix replacement, algorithm optimization, and instrument improvements (Abdelmoula et al., 2018; Alexandrov et al., 2011; He et al., 2015; Morikawa-Ichinose et al., 2019; Song et al., 2017), MSI technology has achieved substantial breakthroughs in its sensitivity, resolution and sample suitability. With the integration of MSI with other technologies (Porta Siegel et al., 2018), such as LC-MS (Desbenoit et al., 2013), microscopic imaging (Tian et al., 2019; Van de Plas et al., 2015), Raman spectroscopy (Bocklitz et al., 2015), and magnetic resonance imaging (Verbeeck et al., 2017), the application of MSI technology to TCMs research will also become broader.

Declaration of competing interests

The authors report no conflicts of interest.

Funding

This work was supported by the National Natural Science Foundation of China [grant numbers 81773996, 81773678, 81973476, and 82074104]; the National Major Scientific and Technological Special Project for “Major New Drugs Development” [grant numbers 2018ZX0973S006, 2018ZX09711001-002-001]; and the Research
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