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Tissue-specific metabolite profiling of alkaloids in Sinomenii Caulis using laser microdissection and liquid chromatography–quadrupole/time of flight-mass spectrometry

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ABSTRACT

Secondary metabolites accumulated in different tissues and cells of herbs are usually bioactive components of herbal medicines. Thus, tissue- and cell-specific phytochemical profiling should be useful for indicating relationship between herbal tissues and chemicals, and evaluating the quality of a medicinal herb. Here, a method that combining laser microdissection and ultra-performance liquid chromatography–quadrupole/time-of-flight mass spectrometry (LMD with UPLC–Q/TOF-MS) was established to achieve simultaneous localization and determination of bioactive components in herbal medicines. Sinomenii Caulis, sourced from the stems of *Sinomenium acutum* (Thunb.) Rehd. et Wils., was set as an illustrative case, and its phytochemicals were profiled by the present method through analyses of different microdissected tissues and cells, involving epidermis, cortex, stone cells, pericycle, vascular bundles and pith. Results revealed that different tissues and cells contained varied alkaloids, among which six alkaloids, i.e. 6-Me-ether-12-O-β-D-glucopyranoside-laudanosoline (peak 4), sinomenine (peak 6), N-norsinoacutine (peak 7), magnoflorine (peak 11), laurifoline (peak 16) and menisperine (peak 17) were detected in all microdissected parts, and sinomenine and magnoflorine were the two most abundant components. By further quantitative determination, alkaloids were generally demonstrated to distribute in the outer part of the cortex, phloem and xylem. According to the relationship between alkaloids and tissues revealed in our study, Sinomenii Caulis of larger diameter has proportionately more bioactive components, and is therefore of higher quality for medicinal use. The method of LMD with UPLC–Q/TOF-MS developed in this study was initially applied to the research of medicinal herbs, and proved to be high sensitive, low cost, convenient and practical.

1. Introduction

It is known that many plants have been put into medicinal applications for thousands of years, and therapeutic effects of medicinal herbs are closely related with their chemical components [1,2], which are mainly secondary metabolites accumulated in tissues and cells of plants [3]. Therefore, the histochemical study on the tissue- and cell-specific chemical profiling is essential and useful for species identification and quality evaluation of the medicinal herbs. And this study is also helpful to understand the distribution rules and accumulation features of bioactive components and to acquire better breeding species of the plant [4].

In previous studies, analysis of plant secondary metabolites has been widely reported [5–11], most of which focused on the detection and determination of the total chemicals in a whole plant by using multiple of modern analytical instruments. For histochemical study, the usual methods were the in situ reaction and determination through chemical and immunological approaches [12,13]. Recently, the technique of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has also been reported, and successfully applied to the metabolite profiling of herbal medicines at tissue level [14–16]. Direct analysis of alkaloids in the tissues of five commonly used Chinese medicinal herbs was conducted for authentication purposes. MALDI is convenient and high throughput since the pre-processing of sample is relatively easy, without separation by liquid chromatography. However, as a coin has two faces, the fast pre-processing before

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analysis makes it provide only accurate mass data but not sequence data, and detect only molecules that can be easily ionized but not exhaustive. Moreover, results are easily influenced by residue of detergent, or any other pollution of sample. Besides, the MALDI imaging is time consuming to get full-scale information about phytochemistry [17–19]. As a result, for the complex plants samples, including various kinds of components, this analytical method is not sensitive enough for phytochemistry profiling in specific tissues or cells, neither can it achieve direct and accurate determination of some major bioactive components at tissue level.

Laser microdissection (LMD), an accurate sampling technique, has been applied to cell separation in life science research for many years [20,21]. In addition to its usual applications in physiological studies [22–26], this technique has been used in plant histochemistry study for single cell capture and analysis [27–29]. In combination with some sensitive detectors, for example, cryogenic nuclear magnetic resonance and mass spectrometry, the chemicals in stone cells of Norway spruce (*Picea abies*) have been analyzed [30], and two novel and one known natural product in secretory tissues were identified [31]. Due to their specific histologic features, both stone cells and secretory tissues were distinguished and then dissected under a light microscopy. With the development of fluorescence microscopy, diverse tissues and cells with ergastic substance in transverse section of plant were investigated, focusing on the feature of chemicals with intrinsic fluorochromes, which have the ability to emit light [32–34]. The fluorescence characteristics of herbal medicines have also been investigated [35–37], demonstrating various fluorescence light in different tissues or cells. Through the accurate dissection technique of LMD, each tissue or cell showing specific fluorescence light which represents certain kinds of constituents can be separated exactly for further analysis.

Ultra-performance liquid chromatography–quadrupole/time-of-flight-mass spectrometry (UPLC–Q/TOF-MS) has the merits of high detection sensitivity and short analysis time, making it a powerful analytical tool for the analysis of trace herbal components [38,39]. The co-eluting peaks can be isolated by mass selectivity and are not constrained by chromatographic resolution. It can generate a chemical fingerprint for the active components and ensure correct peak assignment in the presence of complex matrices. The known and unknown metabolites can be identified by accurate molecular weight and structural information provided by Q/TOF-MS. Moreover, quantitative and qualitative data can be obtained easily with limited instrument optimization.

Sinomenii Caulis (*qingfengteng* in Chinese), stem of *Sinomenium acutum* (Thunb.) Rehd. et Wils, is widely used in China to treat rheumatism, arthromyodynia and similar diseases for over a thousand years [40]. Modern pharmacological studies have demonstrated that its anti-inflammatory and depressurization effects are attributed primarily to its alkaloids in general and to sinomenine in particular [41]. Sinomenine is reported to be generally responsible for the effects of anti-inflammatory, anti-rheumatic, immunosuppressive, anti-hypertensive, anti-arrhythmic, cardiac depressive, anti-angiogenesis, anti-anxiety and vasodilatation, etc. [42–46]. Besides, other alkaloids have also been found to demonstrate some bioactivities, such as analgesia, cell protection and antioxidation [47–49]. However, most of the *S. acutum* available commercially is harvested from the wild. Thus, quality evaluation of the commercial medicinal materials and efficient utilization of the resources of *S. acutum* are of great importance to the present research and applications.

In the present study, laser microdissection guided under fluorescence and coupled with liquid chromatography–mass spectrometry was explored as a new histochemistry method. It was applied to Sinomenii Caulis to give spatial profiling of its major bioactive components through simultaneous quantitative and qualitative analyses. Results provide information about chemical basis of the plant tissues and cells, and also establish a scientific platform for analyzing the active components of other herbal medicines by histochemical approaches.

2. Materials and methods

2.1. Chemicals and reagents

All the reference standards were purchased from authorized companies and units, i.e. sinomenine (abbr. sm) from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing city, China), magnoflorine (abbr. mf) from Phytomarker Limited Corporation (Tianjin city, China), and sinoacutine (abbr. sa) from Jiangxi Herbfine Technology Limited Company (Nanchang city, Jiangxi province, China). The purity of each chemical was >98%. Methanol (AR grade) and acetonitrile (HPLC grade) were purchased from E. Merck (Darmstadt, Germany). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Medicinal materials

Raw materials of Sinomenii Caulis were purchased from Nanchang city, Jiangxi province of P.R. China. The material was authenticated as dried stems of *S. acutum* (Thunb) Rehd. et Wils. by Professor Zhongzhen Zhao, and a voucher specimen was deposited in the Bank of China (Hong Kong) Chinese Medicines Centre of Hong Kong Baptist University. The diameter of the stems chosen for the present study was in the range of 1–2 cm, and divided into two groups as NC-S (less than 1.5 cm in diameter) and NC-L (more than 1.5 cm in diameter).

2.3. Tissue preparation

After softened by infiltrating with water-soaked-non-cellulose paper, materials of Sinomenii Caulis were cut into small sections and embedded into tissue freezing medium (Leica Microsystems, Germany) and then placed on a cutting platform in the cryobar of a cryostat (Thermo Shandon As620 Cryotome, UK) at −20 °C. Serial slices of 30 μm in thickness were cut at −20 °C and mounted directly to PET slides with steel frames (76 mm × 26 mm, 1.4 μm in thickness, Leica Microsystems, Germany).

2.4. Laser microdissection

After the freezing medium was defrosted and coagulated to make slices adhere firmly, the slides were mounted on a Leica LMD 7000 system (Leica, Benshein, Germany). Cuttings were made by a DPSS laser beam at aperture of 1, speed of 3 under a Leica LMD-BGR fluorescence filter system consisting of an excitation filter (blue light), dichromatic mirror (green light) and suppression filter (red light) with intensity of 17%, and auto exposure time mode. Observations were made at 6.3× (Leica 518145, UV|3×/0.13, Germany), 10× (Leica 506507, HCX PL FLUOT AR 10×/0.30, Germany), and 20× (Leica 506243, HCX PL FLUOT AR 20×/0.4, Germany) magnifications, and microdissection was performed at 20× magnification. Tissue parts or cells within an area of around 200,000 μm^2 were determined as the investigated size and dissected separately according to their autofluorescence color, and collected by a cap of 0.5 ml microcentrifuge tube (Leica, Germany), followed by transferring to the bottom of the tube through centrifugation (Centrifuge 5415R, Eppendorf, Hamburg, Germany) for a few minutes till all tissues or cells fell off from the cap.

For raw materials, one whole transverse section composed of all tissue parts obtained through cryostat sectioning was transferred into a microcentrifuge tube followed by extraction.