Quality Control Approaches for Chinese Herbal Medicines

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Abstract: The current official quality control approaches meet the challenges from the complexity of herbal medicines. In fact, any herbal medicines containing numerous unknown components, its curative effect usually depends on the whole of herbal medicines, so it is impossible and unnecessary to qualitatively and quantitatively study every component. By investigating the limitations of current quality control approaches for herbal medicines and the difference and similarity in the chemical substantial style as well as quality control pattern of herbal medicines, a new quality control approach for Chinese herbal medicines should be explored and designed. The combination approach of chemical analysis with bioassay is promising to be developed and employed in order to ensure the safety and efficacy of Chinese herbal medicines.

Key words: bioassay; chemical analysis; combination approach; herbal medicines; quality control

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Introduction

The clinical application history of herbal medicines is longer than 5000 years, and traditional Chinese medicines (TCM) has been employed to some extent in other countries, such as Korea and Japan, and has attracted considerable attention even in European and North American countries (Bent and Ko, 2004). In the *Chinese Pharmacopoeia* Part I, there are 2165 species of crude plant drugs, pieces, vegetables oil, plant extracts, compounds, and single drug preparations recorded (Pharmacopoeia Committee of P. R. China, 2010). It is well known that herbal medicines, either a single herb or herbal compound recipe, is a complex mixture containing hundreds of different chemical constituents which are usually material bases for its therapeutic effects (Xue and Roy, 2003).

Compared with well-defined synthetic drugs, herbal medicines have some significant differences, e.g., the active principles are unknown partially; The availability and quality of raw materials are uncertain; The standardization, stability assessment, and quality control are feasible but not easy (Calixto, 2000). Therefore, quality control of herbal medicine, unlike that of a chemically synthetic drug with high purity, is beyond the applicability of conventional analysis which basically concentrates on single or few marker components (Cheng and Chen, 2003; Liang, Xie, and Chan, 2004). As written in *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines* (World Health Organization, 2000), "Despite its existence and continuous use over many centuries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training, and research in this field have not been accorded due to attention and support. The quality control of herbal medicine is far enough to meet the criteria needed to support its world-wide use. The reasons for the lack of research data are not only due to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine". In general, one or a few markers of active components with pharmacological effect in herbs or herbal mixtures were currently employed for evaluating the quality and authenticity of herbal medicines (Cheng and Chen, 2003; Liang, Xie, and Chan, 2004). However, the evidence is still lacking between the link of this kind of quantitative criteria with the safety and efficacy of herbal

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medicines (Xiao *et al*, 2009). As such, it might not be a good thing when the content of a specific component is high, or a bad thing when the content of that component is low.

Chromatographic fingerprint, as a comprehensive quantitative identification method, showing chemical information of herbal medicines with chromatogram, spectrogram and other graphs generated by chemically analytical techniques, could show not only naturally occurring and bioactive constituents but also their relative abundances. So the chromatographic fingerprint would be more valid and efficient than traditional quality control approaches of herbal medicines. However, the fingerprint has no direct correlation with the safety and efficacy of herbal medicines, either.

In considering this limitation of existing approaches in quality control for herbal medicines, we believe that quality control method based on bioassay for herbal medicines is indispensable (Xiao *et al*, 2010). By investigating the differences and similarities of quality control approaches for drugs, a new quality control strategy should be explored and designed for herbal medicines. It is necessary to combine chemical constituent analysis with bioassay in order to ensure the safety and efficacy of herbal medicines.

Methods

Chemical analysis approach

Chemical analysis is mainly used to study the chemical composition and structure of substances, including both qualitative and quantitative analyses. The former is to determine those elements and compounds that are present in a sample of unknown material and the latter is to measure the amount by weight of each element or compound presented. These analyses are just useful for analyzing the chemical composition, structure, and relative chemical properties. It could not be used for direct bioactivity detection on substances. In other words, this analysis approach is chemically useful but with no biological utility.

According to the *Chinese Pharmacopoeia 2010*, identification and quantification of chemical markers are crucial to the quality control of herbal medicines. There were more than 800 quantitative assays of chemical markers documented in the *Chinese Pharmacopoeia 2010* for the assessment of medicinal plant materials, plant lipids, herbal extracts, and formulations (Pharmacopeia Committee of P. R. China, 2010; Liu *et al*, 2000; 2003). Based on the *Chinese Pharmacopoeia 2010*, many modern instrumental analysis methods have been applied broadly. HPLC is the key method for quality control of herbal medicine. As recorded in the *Chinese Pharmacopoeia 2010*, we grouped the chemical analysis approaches into single compound approach, multi-compound approach, and fingerprint approach.

Single-compound analysis approach

Only one compound is used as detection marker in qualitative and quantitative analysis of this approach, so it is named single-compound analysis approach. This single-compound analysis approach is only suitable for samples containing only one compound or only one compound in which is peculiar and also available as detection marker. In this case, the single compound must be able to represent the whole sample.

In this approach, some useful methods are applied, for example, the frequently used ones are HPLC, GC, CZE, LC-MS, and so on. HPLC is the key method for single-compound analysis as the method has been validated to be reliable and reproducible. However, the presence and the content of a single compound are not sufficient to meet the requirement of quality control of herbal medicines which may contain hundreds of compounds.

Multi-compound analysis approach

This approach is derived from single-compound analysis approach. The single compound in sample to be analyzed is now replaced with multiple compounds. Actually, almost all the instruments used in single-compound analysis approach are available for multi-compound analysis approach. This would make sense as in some samples, there is no compound that could represent the whole sample, but if two or more compounds are selected as a marker together, they could represent the sample. In this approach, in addition to the marker compounds and their contents in tested sample, the content proportion of marker compounds should also be taken into consideration. This approach expands the scope of application of single-compound analysis approach greatly.

Based on the application of multi-compound analysis approach, multiple compounds, instead of

single compound, are recommended for the quality control of herbal medicines. For example, RP-HPLC-DAD was established to simultaneously determine the seven bioactive lignans in *Herpetospermum caudigerum* Wall., namely *ent*-isolariciresinol, dehydrodiconiferyl alcohol, herpetrione, herpetin, herpetetrone, herpetotriol, and herpetal (Cong *et al*, 2008). Alkaloids of more than 60 kinds were detected in this herb by LC-MS and 15 of them were tentatively identified (Wang *et al*, 2009). The multi-compound approach is more scientifically valid than the single compound approach. But still, the limited number of these compounds in herbal medicine could hardly represent all absolutely.

Fingerprint analysis approach

Fingerprint analysis approach is a whole-information fingerprint technique reflecting the chemical composition of researched object. It is mainly aimed at solving the problems which could not be done by single- and multi-compound analysis approaches. This approach is available for whole spectral analysis on various known and unknown substances.

Today, the new generation of herbal quality standard, quality control, and research platform based on chromatographic fingerprint technique are applied by most of researchers in herbal medicine research field. Instead of randomly selecting single component, a few compounds or bioactive constituents for purpose of quality analysis, the new approach by emphasizing on a series of compounds and their proportions provides a more rational solution, that is chromatographic fingerprint analysis for the authentication and quality assessment of herbal medicines. Pattern recognition was also utilized as a powerful classifier for routine quality assurance of herbal products and eventually employed as a regulatory tool for chromatographic fingerprint analysis.

By definition, the chromatographic fingerprint of one herbal medicine is, in practice, a chromatographic pattern from some common chemical components of pharmacological activity and/or chemical characteristics (Yi *et al*, 2006; Bauer, 1998; State Drug Administration of China, 2000). This chromatographic profile should be featured by fundamental attributions of “integrity” and “fuzziness” or “sameness” and “differences” so as to chemically represent the herbal medicine investigated (State Drug Administration of China, 2000; Xie, 2001). As in any herbal medicine and its extract, there are hundreds of unknown components and many of them are in low quantity. Moreover, certain variability exists within the same herbal materials generally (Welsh *et al*, 1996; Valentao *et al*, 1999; Lazarowych and Pekos, 1998). Thus, consideration of multiple constituents and not solely on one or/and two marker components in the quality evaluation should be given for herbal medicines. As regarding to analytical tools, HPLC is now comprehensively used to establish chromatographic fingerprint for quality control of raw herbal products (Zhao *et al*, 2004; Huang *et al*, 2006; Wang *et al*, 2006; Luo et al, 2009). The fingerprint also can be used to evaluate the quality of herbal preparations, which normally contain more than two kinds of herbs (Liu *et al*, 2006). For example, two methods (HPLC-DAD and HPLC-MS) have been developed and used for fingerprint analysis of *Coptidis Rhizoma* (Sheng *et al*, 2006; Tong *et al*, 2008). Infrared spectroscopy (IR) is also an important technique for the quality control of herbal medicine, as it is rapid and reasonably cost-effective; It will play an important role in the quality control of medicinal herbs (Yu *et al*, 2005; Liu *et al*, 2007).

Fingerprint is a powerful tool for the quality control of herbal medicines, but with the disadvantage that could not provide any information about the bioactivity or safety of herbal medicines.

Bioassay approach

Bioassay addresses the quality problem which could not be solved by using chemical analysis method. All the chemical analysis approaches are only available for the determination of chemical composition, structure, and properties of tested samples, but their biological properties could not be assessed. The importance of bioactivity analysis exists in its direct link to efficacy or safety. It is thus necessary to combine it with advanced chemical analysis approaches for herbal medicines.

In the guide of the *Chinese Pharmacopoeia 2010*, it states that the quality control of herbal medicine should be changed from chemical analysis to bioactivity analysis. Bioactivity analysis is playing a more and more important role in the quality control of herbal medicines nowadays. There are some methods used in bioassay, e.g. antifebrile, anti-inflammation,

antibacterial, antivirus, and so on. The analytical methods for quality control of herbal medicines should have the following attributes: selectivity, accuracy, sensitivity, precision, and suitability. In recent years, we have applied some methods to assess the biological activity of herbal medicines. For example, *Isatidis Radix*, the root of *Isatidis indigotica* Fort., is mainly distributed in Hebei, Jiangsu, Zhejiang, Fujian, Henan, and Guangxi Provinces of China. As a medicinal herb, it has been used for more than 2000 years with the function of preventing and curing diseases. It has strong activities in clearing away heat, cooling blood, removing toxin, and eliminating inflammation. The *in vitro* anti-viral activities of *Isatidis Radix* and its extract on neuraminidase (NA) were assayed by using chemi-luminescence method for the purpose of developing quality control approach for antiviral drugs (Li *et al*, 2009a; 2009b). A method for determination of antiviral activity of *Isatidis Radix* based on the red blood cell agglutination was established according to the positive correlation ($P < 0.01$, $R = 0.83$) between the antiviral activity of *Isatidis Radix* and the agglutination activity of red blood cells. The thrombotest on the microdosis panel was implemented for this method. We also applied microcalorimetry and thermoanalysis as quality assessment techniques for *Isatidis Radix*, as they are playing an increasingly important role in life sciences (Zhao *et al*, 2006). *I. indigotica* had some inhibitory action on *Staphylococci* and *Streptococcus hemolyticus* and inhibitory effect against HBsAg and sub-influnza virus A. Its active component and indirubin had significant bioactivity against leukaemia. We established a novel preliminary screening model for bioactive parts in the leaves of *I. indigotica* based on biothermodynamics, and the conclusion is that its decoction and water-extract stimulate the metabolism of *Escherichia coli*. Other four kinds of organic solvent extracts exhibited the inhibition for the growth of tested bacteria. The microcalorimetric method has many virtues, such as extensive applicability, convenience, *etc* (Wu *et al*, 2006; Zhao, Wang, and Xiao, 2007). We also evaluated the quality of *Isatidis Radix* based on the analysis of biothermodynamic activity. According to our research, we knew that the quality of *Isatidis Radix* was partially related to the content of organic acids, but it could not be correctly recognized by both chemical determination and HPLC fingerprint. By contrast, the mathematic model based on four main parameters of biothermodynamic analysis was very suitable (misjudgement ratio of 1.39%) for evaluating the quality of *Isatidis Radix* (Zhao, Wang, and Xiao, 2007). *Coptis Rhizoma* has been widely used to treat a vast array of health problems and medical conditions. The same method as treated for *Isatidis Radix* was used to assay berberine alkaloids in *Coptis Rhizoma* based on antibacterial potency, which is also fast and suitable (Yan *et al*, 2008). It could imply the possibility of using this method in a wide range of drugs to determine their antibiotic activity and supply these thermograms as a "fingerprint" to research the bioactivities of drugs. For some herbal drugs, the correlation between their bioactivities and therapeutic effects is clear, and the bioassay would work as a mean of quality control.

Combination approach of chemical analysis with bioassay

Based on the establishment of relative biochemical and physiological substance index and index system, the advanced chemical analysis approaches are to be combined with bioassay. Essentially, all the bio-activities of substances are relative to their chemical composition, structure characteristics as well as properties. As such, the chemical and biological properties of known and unknown substances could be studied together. This new analysis approach is particularly applicable for studying Chinese materia medica, herbal medicine as well as traditional remedies from other countries.

The quality control of herbal medicines will be shifted from single-compound-index analysis to combination of active components analysis with bioassay, and the latter has been explored in our institute. From the characteristic of herbal medicines we know that their chemical compositions are complex and diverse. We also know that the therapeutic effects of the compounds extracted from herbal medicines may have different functions and mechanisms, and there are more similarities in chemical substantial style between Chinese herbal medicines and biologicals than that between Chinese herbal medicines and chemical synthetic drugs. A new quality control pattern of herbal medicines should be established on the basis of combination approach of chemical analysis with

bioassay. Chemical analysis is mostly used for assessing compounds, while the bioassay is mostly used to target biological effects (antibacterial, antivirus, and so on). The definition of profile-effect, spectrum-effect, or fingerprint-efficacy was applied in combination approach for chemical analysis with bio-assessment. In recent years, we investigated the spectrum-effect relationships between HPLC fingerprints and the antibacterial activities of EtOAc extracts from *Radix Isatidis*. Fingerprints of EtOAc extracts of *Isatidis Radix* from various sources were established by HPLC. The growth process of *E. coli* affected by EtOAc extracts was monitored by using Thermal Activity Monitor (TAM) Air Isothermal Calorimeter and microcalorimetry. This work provides a general combination model of HPLC with microcalorimetry for studying the spectrum-effect relationships of EtOAc extracts from *Isatidis Radix*, which can be used to screen principal components of *Isatidis Radix* on the basis of bioactivity (Kong *et al*, 2008a). We also evaluated the spectrum-effect relationships between HPLC fingerprints and biothermodynamic activity of *Zuojinwan* and its similar formulas. There was close correlation between the spectrum-effect relationships based on the HPLC fingerprints and biothermodynamic activity (Kong *et al*, 2008b). Shen *et al* (2008) had demonstrated that fingerprints correlated with pharmacodynamic effects of bioactive compounds in *Paeonia lactiflora* Pall and *Glycyrrhiza uralensis* Fisch, and this study provided a valid approach for understanding the material foundation of traditional Chinese herbal medicine. The corresponding relationship between fingerprints and effects of traditional Chinese herbal medicine can be used to control the quality of both herbal medicine and formulae (Lu, Liang, and Qian, 2005; Lu *et al*, 2006).

In summary, the combination approach of chemical analysis with bioassay is a practicable and comprehensive approach for the quality control of herbal medicines. Such tools showed prosperous prospect for pharmacists and analytical chemists to directly address challenging problems in quality control of herbal medicines.

Results and discussion

It is well known that herbal medicines possess complex mixture of chemical compounds, thus reasonable evaluation of their relationship is very important. The combination approach of chemical analysis with bioassay may provide a relatively complete quality control approach. By investigating the limitation of quality control pattern for herbal medicines, the differences and similarities in chemical substantial style as well as quality control methods among herbal medicines, a new quality control strategy for herbal medicine should be explored and designed. The combination approach of chemical constituent analysis with bioassay should be developed and employed in order to ensure the safety and efficacy of herbal medicines.

In some situations, the correlation between bioactivity and therapeutic effect of drugs is clear and their chemical compositions are basically known. This combination approach will be more applicable and meaningful. While in other occasions, the correlation between bioactivity and therapeutic effect of drugs is not clear, their chemical compositions are unknown, or their therapeutic effect is uncertain, this combination approach may not be practically applicable.

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