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Review

Advanced strategies for quality control of Chinese medicines

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ABSTRACT

Quality control is always the critical issue for Chinese medicines (CMs) with their worldwide increasing use. Different from western medicine, CMs are usually considered that multiple constituents are responsible for the therapeutic effects. Therefore, quality control of CMs is a challenge. In 2011, the strategies for quantification, related to the markers, reference compounds and approaches, in quality control of CMs were reviewed (Li, et al., J. Pharm. Biomed. Anal., 2011, 55, 802–809). Since then, some new strategies have been proposed in these fields. Therefore, the review on the strategies for quality control of CMs should be updated to improve the safety and efficacy of CMs. Herein, novel strategies related to quality marker discovery, reference compound development and advanced approaches (focused on glyco-analysis) for quality control, during 2011–2016, were summarized and discussed.

Contents

1. Introduction

Quality control, including qualitative and quantitative analysis, is always crucial for ensuring the safety and efficacy of Chinese medicines (CMs). For quantitative analysis, three aspects, i.e. quality marker, reference compound and approaches, should be considered [1]. Generally, quality markers should be closely related to the safety and efficacy of the CMs, which should be changed based on the therapeutic purpose of a CMs. Taking *Panax notoginseng* as an example, the quality marker of dencichine is used for hemostasis but saponins are used for treatment of cardiovascular diseases (USP-Herbal Medicines Compendium, https://hmc.usp.org/monographs/panax-notoginseng-root-and-rhizome-1-0). While reference compounds should be enough for meeting the development of quality control of CMs, or else, the alternative strategies should be proposed. Especially, sample preparation method for quality control of CMs should reflect their characters and advanced techniques are usually necessary to improve the specificity and accuracy of quantification. In this article, novel strategies related to quality marker discovery, reference compound development and advanced approaches (focused on glycol-analysis) for quality control were summarized and discussed.

2. Discovery of quality markers

Effective components are usually used as quality markers for CMs, which include active and relative components [1]. Active

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components are the chemical compounds with specific biological activity related to the therapeutic effects of CMs. Relative components are the compounds which may have no specific action related to the efficacy but can definitely affect the therapeutic effects of active components in CMs. A series of ethanolic and methanolic extracts of St. John's wort were investigated and, in almost all cases, the extracts showed strong antidepressant activity. Only one methanolic research extract had no effect in the *in vivo* pharmacological experiments. HPLC characterization showed that the inactive extract had a reduced level of rutin, though rutin alone had no antidepressant activity. It is interesting that addition of rutin to the inactive extract, to produce a concentration within the normal range, resulted in a strong pharmacological effect comparable to that of the other extracts. The studies suggested that this re-activation was not dose-dependent, indicating that rutin must be present above a threshold limit. It therefore appears vital that extracts of St. John's wort which are designed for the therapy of depressive disorders should be manufactured using plant material with sufficient amounts of rutin [2]. Indeed, *Hypericum* species showed that there were chemotypes and varieties which contained no rutin, and the rutin contents was dependent on the altitude of the grouwing site [3–5].

In last decades, the techniques for screening and identification of active components, i.e. the techniques for discovery of quality marker, in CMs have been developed considerably. Different from conventional methods, i.e. systematic separation followed by bioassay and bioassay guided separation approaches, chromatographic separation coupled with biochemical detection has been widely used for screening active components in CMs [1,6]. The inhibition of xanthine oxidase (XO) is recognized as one of the therapeutic approaches to treat gout. A post-column continuous XO inhibition assay coupled to HPLC separation and diode array detection, as well as MS/MS system was developed for the screening of XO inhibitors. The availability of the online system was first tested with a positive drug, allopurinol, a well-known XO inhibitor, and subsequent analysis of *Scutellaria baicalensis* extract showed that two main bioactive compounds with XO inhibitory activities were observed, indicating that the developed online system was applicable to complex mixtures [7]. Though chromatographic separation coupled with biochemical detection is a powerful technique, drug discovery from complex mixture like Chinese herbs is still a challenge and extensive false positives make the obtainment of specific bioactive compounds difficult. A novel sample preparation method was proposed to rapidly reveal the specific bioactive compounds from complex mixtures using α-glucosidase as a case. Four extracts out of aqueous and methanol extracts of 500 traditional Chinese medicines, i.e. fruit of *Terminalia chebula* (FTC), flowers of *Rosa rugosa* (FRR) and *Eugenia caryophyllata* (FEC) as well as husk of *Punica granatum* (HPG), showed high inhibition on α-glucosidase. On-line liquid chromatography–diode array detection–tandem mass spectrometry and biochemical detection (HPLC–DAD–MS/MS–BCD) was performed to rapidly screen and characterize α-glucosidase inhibitors in these four extracts. After tentative identification, most of compounds with inhibitory activity in the investigated crude extracts were found to be tannins commonly recognized as non-specific enzyme inhibitors *in vitro*. Therefore, the four extracts were treated with gelatin to improve specificity of the on-line system. Finally, two compounds with specific α-glucosidase inhibition were identified. The developed method could discover specific α-glucosidase inhibitors in complex mixtures such as plant extracts, which could also be used for discovery of specific inhibitors of other enzymes [8]. XO inhibitors and free radical scavengers are beneficial to the treatment of gout and many related diseases. An on-line HPLC coupled with post-column dual-bioactivity assay was established and successfully applied to simultaneous screening of XO inhibitors and free radical scavengers from a complex mixture, *Oroxylum indicum* extract [9]. Recently, the online strategies, which integrated the separation science, mass spectrometry, and bioactivity screening in a single platform, allowing simultaneous screening and characterization of active compounds from complex matrices, especially from the herbs were reviewed [6]. The online screening methodologies, including pre-column affinity-based screening and post-column bioassay, were discussed and their applied examples were also presented to illustrate the strengths and limitations of these approaches. Besides, an overview of the current advances in online two-dimensional-based cell membrane chromatography for screening target components from traditional Chinese medicines has also been summarized [10]. Actually, recent developments in the use of analytical techniques for the identification of novel active components from plant extracts including advanced hyphenated techniques in phytochemical analysis, ultrafiltration HPLC–MS, on-line HPLC-biochemical detection, cellular membrane affinity chromatography and ligand fishing using protein coated magnetic beads were reviewed [11]. The limitations and advantages of each of these methods have also been discussed. Nevertheless, there are many methods for discovery of active components in CMs [6,10,11]. However, few approaches were proposed for discovery of the relative components though the holistic function of CMs has been widely known and relative components of CMs may contribute a vital role in the efficacy of active components [2,12–14].

The component-effect correlation analysis, combination of comparative chemistry and comparative biological activity are helpful methodologies [1]. The chemistry and toxicology between ethanol and water extracts of *Polygonum multiflorum* were investigated and compared. The results showed that ethanol extract had much stronger hepatotoxicity than that of water extract, and the contents of emodin-8-*O*-β-D-glucopyranoside, physcion-8-*O*-β-D-glucopyranoside, emodin and physcion were significantly higher in ethanol extract than in water extract, which suggested these compounds might be the potential hepatotoxic components. Human hepatocytes extraction also showed that 2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside, emodin-8-*O*-β-D-glucopyranoside, physcion-8-*O*-β-D-glucopyranoside, emodin and physcion had interaction with human hepatocytes. The hepatotoxic effect of these components was investigated on human hepatocytes LO_2 cells and emodin-8-*O*-β-D-glucopyranoside, physcion-8-*O*-β-D-glucopyranoside, emodin and physcion were finally confirmed to be, at least partially, the hepatotoxic components [15]. Using *Colubrina greggii* as a model, chemometric analysis combined with data from a leishmanicidal bioassay were also used to detect biologically active natural products in crude extracts from plants having little or no phytochemical information. A first analysis of the HPLC-UV profiles of the extract and its semi-purified fractions using both principal component analysis (PCA) and orthogonal partial least squares (O-PLS) indicated that the components at t_R 48.2, 48.7, 51.8 min correlated with the variation in bioactivity. However, a further O-PLS analysis of HPLC-UV profiles of fractions obtained through a final semi-preparative HPLC purification showed two components at t_R 48.7 and 49.5 min which correlated with the variation of the bioactivity in a high performance predictive model had high determination coefficient, high correlation coefficient values and a low root mean square error. This study demonstrated that the association of chemometric analysis with bioassay results could be an excellent strategy for the detection and isolation of bioactive/bio-relative metabolites from phytochemically unknown plant crude extracts [16]. In addition, a novel strategy was proposed to determine effective components (active and relative compounds) which were responsible for the holistic bioactivity of an herbal product. To support the strategy, the pharmacophore-guided knockout/knockin chromatography was established. The greatest advantage of this method is that any

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