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Characterization of the therapeutic properties of Chinese herbal materials by measuring delayed luminescence and dendritic cell-based immunomodulatory response

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ABSTRACT

Based on the traditional Chinese medicine theory, the Chinese pharmacopeia assigns a therapeutic description of "taste" to all herbs; thus, an herb's "taste" is valued in traditional Chinese medicine as a major ethnopharmacological category and reflects the herb's therapeutic properties. These properties guide the practitioner with respect to preparing a specific herbal formula in order to provide each patient with a personalized intervention. The key challenge in evidence-based medicine is to characterize herbal therapeutic properties from a multi-target, multi-dimensional systems pharmacology perspective. Here, we used delayed luminescence (DL, the slowly decaying emission of photons following excitation with light) as a rapid, direct, highly sensitive indicator to characterize the properties of herbal medicines. The DL parameters were able to reliably identify a specific category of herbal materials with the so-called "sweet" taste. To support the DL results and provide biological relevance to the DL results, we used a murine bone marrow-derived dendritic cell-based assay to examine the immunomodulatory effects of herbal extracts from various "taste" categories. Our results indicate that DL may serve as a robust and sensitive tool for evaluating the therapeutic properties of herbs based on the traditional Chinese medicine classification of "taste". Thus, DL provides a promising technological platform for investigating the properties of Chinese herbal medicines both qualitatively and quantitatively.

1. Introduction

Chinese herbal medicine has been used in China for thousands of years to maintain health and treat disease [1]. In this long history, practitioners of traditional Chinese medicine have accumulated a wealth of knowledge regarding herbal therapeutic effects based on clinical observations, resulting in the classification of herbal medicines into specific therapeutic categories [1,2]. Chinese herbal medicines are traditionally classified according to the sensations they evoke and the patient's response, resulting in descriptive characterizations such as taste, warm/cold, and toxic/non-toxic [1–3]. The taste category includes descriptors based on the perception in the mouth and include sweet, bitter, pungent, salty, and sour [4]. Our current understanding of the herbal descriptor "taste" developed from a long history of clinical experience and is linked to specific therapeutic properties in humans [3,4]. Hence, in traditional Chinese medicine, herbs are not necessarily classified according to their perception in the mouth, but rather according to their therapeutic properties in the human body [4]. Thus, the five taste descriptors have been used to classify the specific therapeutic properties and pharmacological actions of Chinese herbal medicines in clinical practice [5]. As a result, the taste descriptors of some herbal medicines currently described in the herbal materia medica can differ from how they actually taste in the mouth [6].

Based on the classification of taste in traditional Chinese medicine, herbs with the same taste descriptor generally possess similar therapeutic properties, and herbs with different taste descriptors generally have different therapeutic properties [6]. For example, the so-called "sweet" class of herbs is associated with a tonic effect that can nourish

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the body, hence promoting a healthy status, boosting the immune system, and helping fight the aging process [4,7,8]. On the other hand, herbs in the so-called "pungent" class eliminate pathological agents and therefore treat the corresponding symptoms (e.g., stagnation) by promoting the circulation of energy and blood [4]. Herbs in the so-called "bitter" class have heat-cleansing and detoxification effects and are used to treat constipation, inflammation, infection, and other conditions [5,9].

Herbs that have a single taste descriptor generally have basic therapeutic properties. However, many herbs belong to two or more taste classes and therefore have a wider therapeutic range than herbs with a single taste descriptor [5]. These taste descriptor–based therapeutic classifications are often used to help the practitioner prepare specific herbal formulas in order to achieve personalized intervention [1]. Therefore, the taste descriptor is an important concept for understanding an herb's therapeutic effect and clinical application, and it is listed both in Chinese medicine textbooks and in the Chinese Pharmacopoeia [10,11]. Interestingly, traditional Indian medicine (i.e., Ayurveda) also uses taste descriptors to indicate the pharmacological activity of herbal medicine [12,13]. Thus, both Ayurveda and traditional Chinese medicine use a common system of taste descriptors. In addition, statistical analyses support the use of herbal taste descriptors for predicting the pharmacological activity of herbs [12]. However, evidence-based scientific data is still needed in order to understand the therapeutic properties of herbal medicines based on taste descriptors.

Delayed luminescence (DL) is the long-term decay of weak photon emissions from materials following exposure to light with a wavelength of 400–800 nm [14]. DL provides a holistic, integrated, comprehensive method for measuring materials and biological systems, and provides a direct, rapid, and sensitive indicator of a wide range of processes, including food quality, seed germination, and cancerous cells [15–17]. Recently, we used DL to study the features of dry powders prepared from Chinese herbal materials [14,18]. The results suggest that specific DL properties can be used to indicate differences in herbal materials prepared under different conditions, including the processing method and the age of the herb [18]. Importantly, DL can be used to detect differences in the overall signatures of a given herb grown under various environmental conditions, and distinct DL properties have been correlated to the specific bioactive constituents extracted from these herbal samples [14]. These differences in DL properties indicate the presence of different bioactive constituents as a result of environmental factors [14]. Because these studies confirm that DL can reflect herbal characteristics at the systems level [14], we hypothesized that DL may be used to increase our understanding of herbal therapeutic properties based on the taste descriptor.

Here, we measured DL in Chinese herbal materials with different taste descriptors. In addition, to support our finding of distinct clusters of sweet descriptors and other herbal taste descriptors based on our DL data, we used murine bone marrow-derived dendritic cells (DCs) in an assay to examine the immunomodulatory effect of herbal extracts from various taste descriptor categories. DCs are specialized leukocytes that play a key role in initiating the adaptive immune response [19], and the production of cytokines such as TNFα and IL-6 by DCs has been used as an indicator of the immunomodulatory capacity of herbal medicines [20]. The results obtained with our DC-based immunomodulatory assay generally support the results of our DL experiments; therefore, DL may provide both qualitative and quantitative insights into the therapeutic properties of herbal medicines.

2. Materials and Methods

2.1. Herbal Materials

A total of 90 herbal materials (roots and/or rhizomes), ginseng leaves, and ginseng flowers were purchased in five batches from TongRenTang Co., Ltd. (Beijing, China). These 90 herbal medicines are listed in Table 1 and are classified according to six taste descriptor groups—sweet, bitter, pungent, sweet & bitter, sweet & pungent, and bitter & pungent—in accordance with the 2015 Chinese Pharmacopoeia [11]. The identities of all herbal samples were verified by Dr. Wen-Te Chang (China Medical University) and were deposited at China Medical University (Taichung, Taiwan).

2.2. Delayed Luminescence (DL)

2.2.1. Sample Preparation

Each herbal sample was crushed using a model QE-100 grinder (Yili Company, Zhejiang Province, China) and passed through a standard sieve to obtain150-μm particles [18]. These herbal samples were kept in a dark, light-tight box containing 35-mm silica gel (Boom BV, Meppel, the Netherlands) at room temperature for 16 h before DL measurements were conducted [18].

2.2.2. DL Measurement

DL was measured in the herbal samples as described previously [18]. The instrument used to measure DL was obtained from Meluna Research (Geldermalsen, the Netherlands) and included a photomultiplier tube (PMT) (type 9558QB; Electron Tubes Enterprises Ltd., Ruislip, UK) vertically positioned on a dark sample chamber kept at 22 °C. The PMT contains a cathode end (51 mm diameter) with sensitivity at 160–870 nm. The PMT was cooled to −25 °C in order to reduce the dark count rate to 10 counts per second. The DL signal was amplified using a type 9301 fast preamplifier (ORTEC, Oak Ridge, TN). Data were acquired using a personal computer containing a model 6602 counting card (National Instruments, Austin, TX). Each herbal sample (1 g) was placed in a plastic Petri dish (35-mm diameter) and excited for 10 s using a model 284–2812 white halogen light source (Philips, Germany). For each sample batch, the DL signal was measured three consecutive times, and a total of fifteen measurements in five batches were used to examine the DL properties of that particular herbal medicine. The DL decay signature was obtained by recording the number of photon counts in consecutive 0.05-s periods for a total of 60 s, yielding a total of 1200 data points.

2.3. Dendritic Cell Assay

2.3.1. Preparation of Extracts

Sixteen herbal samples were tested using the DC assay (Table 1). Each herbal sample was divided into three batches, and each batch of a specific herb was used to prepare four 4-g samples; hence, each herbal sample was divided into 12 independent samples, which were crushed into powder form using a type QE-100 grinder (Yili Company, Zhejiang Province, China). Each powdered herbal sample was extracted with 80 ml distilled water at 100 °C for 1 h. The resulting extraction was then centrifuged, filtered through No. 1 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and concentrated using a model R-210 rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland) [20]. Thereafter, the water extract was mixed with 95% ethanol at a ratio of 1:5.7 (v:v), chilled at 4 °C overnight, and then centrifuged at 5000 rpm for 30 min to precipitate polysaccharides and proteins. The resulting supernatant was then filtered through No. 1 filter paper (Toyo Roshi Kaisha, Ltd.). Finally, 12 independent water extracts were obtained from each original herbal sample, dried in a vacuum evaporator overnight, and stored at 4 °C until further use in the DC assay.

2.3.2. Mice

ICR mice (8–12 weeks of age) were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). All animals were housed in a specific pathogen-free facility at the Animal Center of China Medical University (Taichung, Taiwan) and handled in accordance with the Institutional Animal Care and Use Committee of China Medical University (Taichung, Taiwan) [20].