OPEM
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Oriental Pharmacy and Experimental Medicine 2008 **8(2)**, 103-110
DOI 10.3742/OPEM.2008.8.2.103

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Review

Evidence-based herbal medicine in efficacy and safety assessments

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Received for publication March 07, 2008; accepted May 01, 2008

SUMMARY

Herbal medicine is the use of medicinal plants for prevention and treatment of diseases: it ranges from traditional and popular medicines of every country to the use of standardized and tritated herbal extracts. Generally cultural rootedness enduring and widespread use in a traditional medical system may indicate safety, but also efficacy of treatments, especially in herbal medicine where tradition is almost completely based on remedies containing active principles at very low and ultra low concentrations or relying on magical-energetic principles. The efficacy and safety assessments of medicines, whether modern or herbal, invariably encounter challenges or problems during the course of pre-clinical and clinical research. Some of the challenges in evidence-based herbal medicinal research are unique, and the researcher must be cognizant of them in order to safeguard the quality of the data obtained. Key challenges are: the quality of raw materials; appropriateness of biological/pharmacological activity assessment methodology, and data interpretation; standardization methodology; pharmacokinetics and bioavailability of active constituents and metabolites; clinical dosage formulation/production; and clinical study designs and outcome measures.

Key words: Herbal medicine; Efficacy and safety assessment; Evidence based medicine

INTRODUCTION

Herbs are natural products and their chemical composition varies depending on several factors and therefore varying from people, from energetic decoctions to the use of herbal extracts following Western methodologies of mainstream medicine. Traditional medicines has a very long history: it is the sum total of the practices based on the theories, beliefs and experiences of different cultures and times, often inexplicable, used in the maintenance of health, as like in the prevention, diagnosis, improvement and treatment of illness.

Over the past two decades, the use of alternative and complementary therapies, particularly botanical supplements, has become a topic of increasing global importance with both medical and economic implications (Mahady, 2001). According to a 1983 World Health Organization (WHO) estimate, a majority of the population in developing countries depend on traditional and herbal medicines as their primary source of health care (Bannerman *et al.,* 1983). In a 1997 survey, American consumers spent an estimated US$ 5.1 billion on herbal

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medicines, primarily in the form of dietary supplements (Eisenberg *et al.,* 1998). Even as the use of botanical products has increased dramatically in recent years, either in the form of drugs in traditional systems of medicine (TRM) such as Traditional Chinese Medicine (TCM) or as botanical dietary supplements in Complementary and Alternative Medicine (CAM), the evidence of their efficacy and safety has not been well documented. If these preparations are continued to be used in TRM or CAM therapy, they should be developed as "evidence-based medications" relative to their efficacy and safety.

Evidence of efficacy and safety of herbal medicines, like all forms of medicaments, can best be generated by clinical studies under Good Clinical Practices (GCP). Unfortunately, very few such studies on herbal products have been published. In the past two decades, most clinical studies have been conducted in Europe on single herb preparations, with very few being concerned with herbal mixtures such as those commonly found in TCM. With respect to the latter, a review of 2938 RCTs on TCM reported in 28 journals in the period of 1980 - 1997 (Tang *et al.,* 1999) concluded that the majority of these studies suffered from methodological defects. Additionally, these studies, as well as other clinical studies on herbal medicine have been conducted by clinicians, who, being accustomed to evaluating pure single molecule drugs, are unaware that the assessment of efficacy and safety of herbal products in basic and/or clinical studies can be severely hampered by a lack of appropriate quality assurance or quality control in the preparation of herbal extracts (Fong, 2002; Barrett, 2004; Tyler, 2004). Consequently, current evidence-based herbal medicine studies suffer from the duo challenges of GCP and quality assurance of the study product in general. However, it should be noted that even when studies are designed to incorporate GCP employing well characterized and standardized herbal preparations, other less obvious, but nevertheless, important problems of challenges abound that require attention.

Herbal medicinal products

There is good evidence from systematic reviews/ meta-analyses (including Cocharne reviews) of randomized controlled trials for the efficacy of certain standardized herbal extracts in particular clinical conditions, e.g. standardized St John's Wort extracts in relieving symptoms of mild-to-moderate depression (Linde and Mulrow, 2003), saw plmetto extracts in treating symptoms of benign prostatic hyterplasia (Ernst and Pittler, 1999) and standardized ginkgo leat extracts in symptomatic relief of cognitive deficiency and dementia (Wilt *et al.,* 2003).

In some cases, further clinical trials have been carried out since these systematic reviews were published. For example, recent studies have generally confirmed that standardized St John's Wort extracts are more effective than placebo in mild-to-moderate depression and have provided some evidence that such extracts may be as effective as certain conventional antidepressant drugs at the lower end of the therapeutic range. However, further rigorous randomized controlled trials are required to confirm these effects and to test the efficacy of numerous other herbal medicines for which there is little or no clinical evidence.

Although rigorous clinical investigations are lacking at present for many herbs, there is a vast literature on the phytochemistry and *in vitro* and *in vivo* pharmacological effects of medicinal plants (Schulz V *et al.,* 2000). This information affords a rationale for further investigation of such plants and provides supporting data where clinical evidence exists.

Pragmatic studies for efficacy and effectiveness in traditional medicine

Pragmatic trials (PT) are designed to find out about how effective a treatment actually is in everyday practice; while explanatory trials are designed to

find out whether a treatment has any efficacy, almost always compared with placebo under ideal conditions. PT answers questions about the overall effectiveness of an intervention and cannot study the contributions of its different components. The participant to these studies will need to be representative of the wider population because results need to be generalized; so wide criteria of inclusion are needed, so that patients having more medical diseases or taking different medications are included. It would be more satisfactory and sensible to choose conditions where conventional treatment is often unsatisfactory like irritable bowel syndrome or panic crises. In PT it is not usually mandatory to use a placebo, while it is needed with both arms of the trial on normal practice, since the aim is to produce an evidence to facilitate a real practical choice. The treatment protocol is included, so is necessary a larger sample of patients, and may need a handbook that defines parameters for treatment (Allen *et al.,* 1998). The main advantage of PT is that they can deliver evidence of effectiveness directly in clinical practice. Nevertheless they have important methodological limits: most of all the lack of placebo and blindness, increased costs, the need of several therapists, more complexity and lack of clarification about the mechanism of action; but PT should be seen not as an alternative to explanatory studies, but as a mandatory complement that define and improve evidence primary coming from explanatory trials, the only on that can reliably confirm efficacy.

The chemical constituents of herbal remedies

The other black box of herbal-based treatments is the lack of information about the composition of the remedy. Herbs are natural products and their chemical composition varies depending on several factors, such ac botanical species, used chemotypes, the anatomical part of the plant used (seed, flower, root, leaf and so on) and also storage, sun, humidity, type of ground, time of harvest, geographic area; and merchandised products containing on the label the same product varying in their content and concentrations of chemical constituents from batch to batch; and even the same manufacturer can merchandise in different periods products containing different substances although standardized to achieve a high pharmaceutical quality. This variability can result in significant differences in pharmacological activity: involving both pharmacodynamics and phamacokinetics issues.

Adverse and side effects in another open problem, because in citizens still prevail the respect for everything that is natural tout court, more as a cultural-fashion-based choice than thinking that the patient is introducing in his/her body chemical substances of vegetal origin; not knowing that salicylic glucosides and lactonic sesquiterpenes of many Compositae are often responsible of allergic reactions; that some constituents of plant are cancerogenic like safrole, bergapten and pyrrolizidines alkaloids. Not of minor importance especially for the old patient using contemporary more synthetic drugs is the problem of drug interferences; some plant reduce or improve the bioavailability of some drugs due to induction or inhibition of cytochromes (St. John's Wort extracts, grapefruit juice and so on). Moreover the use of herbal extracts during pregnancy or lactation should undergo strict medical supervision because many herbs have not been studied neither in pregnant mice.

Advances in high-throughput experimentations have resulted in massive datavases of genomic, proteomic and chemical data which in combination with efficient separation methods and powerful spectrometric methods for identification and structure elucidation can be used for identification of active compounds (Chavan *et al.,* 2006). A powerful and deep biological approach that integrates such large and diverse sources of information together actually needs to fully understand the pharmacological effects of natural products; and DNA microarrays may provide a suitable high-throughput platform for research and development of drugs from natural products (Chavan *et al.,* 2006). There are

three main applications of DNA microarrays: in pharmacodynamics for discovery of new drugs; in pharmacogenomics for discovery of new drugs; in pharmacogenomics for prediction of side-effects; in pharmacognosy for correct botanical identification and authentication of crude plant materials as part of standardization and quality control (Chavan *et al.,* 2006).

Selection of appropriate bioassays

The use of an appropriate biological/pharmacological activity assessment methodology is self-evident, whether it is employed in drug discovery research or for determining the mechanism of action of a herbal medicine. In the case of T. pratense, it has been well established that this herb contains oestrogenic isoflavones. Indeed, a MeOH extract of our source material was confirmed to be oestrogenic *in vitro* and *in vivo* (Liu *et al.,* 2001; Burdette *et al.,* 2002). On the other hand, the mechanism of action in reducing hot flashes by C. racemosa was not well understood at the time of our initial study on this herb. A review of the literature (Mahady *et al.,* 2002) cited earlier and contradictory reports of whether there was an oestrogenic effect. In our hands, a MeOH extract of our source material was not oestrogenic (Liu *et al.,* 2001; Burdette *et al.,* 2003), hence the employment of these assays would be inappropriate. Consequently, it was necessary to explore other mechanisms for its hot flash reduction effect. At the end, we discovered that a 40% 2-propanol extract inhibited serotonin receptor. Specifically, it inhibits the 5-HT_7 and 5-HT_{1A} receptors (Burdette *et al.,* 2003). The discovery of this centrally acting mechanism allowed us to overcome a major hurdle to our chemical, biological and clinical studies of black cohosh.

Bioassay-directed isolation bioactive marker(s)

The major challenge in conducting bioassay-directed isolation of bioactive marker compound or compounds lies not in the actual phytochemical isolation process itself, nor in the chemical characterization/structure elucidation. With an array of separation techniques including adsorption, size-exclusion, counter current partition and preparative high-performance liquid chromatography, the isolation of phytochemical constituents has become a matter of routine. With the availability of increasingly more powerful nuclear magnetic resonance and mass spectrometric (MS), and other spectroscopic instrumentation, the characterization, structure identification and/or structure elucidation of isolated molecules can be accomplished with greater and greater ease.

Instead, the major problem encountered in our studies is a biological one. The isolation of actein, 26-deoxyactein and 23-epi-26-deoxyactein as the bioactive markers from C. racemosa (Chen *et al.,* 2002) was complicated by the time consuming process of identifying the HT_7 receptor inhibition assay as the appropriate bioassay to be used in guiding the isolation of these compounds. Once the appropriate bioassay was identified, the phytochemistry and structural identification/elucidation work proceeded without major problems.

Standardization of pre-clinical and clinical extract

As indicated above, the research philosophy of our Center is that any botanical supplement/herbal medicine to be considered for clinical trial studies must be chemically and biologically standardized. Having determined the biological mechanisms of action and through bioassay-directed isolation procedures that identified the bioactive marker compounds (Liu *et al.,* 2001; Burdette *et al.,* 2002, 2003), the next step was to chemically analyse and standardize these extracts to contain the appropriate concentrations of the active compounds for subsequent pre-clinical and clinical studies. Procedures for the quality control analysis of active marker chemical compounds can be accomplished by colorimetric, spectroscopic and/or chromatographic methods. Colorimetric and direct spectroscopic methods are older analytical procedures quantifying the absorption of structurally related compounds

at a specific wavelength of light, and expressed as concentration of a reference standard (marker). Since other plant constituents possessing the same absorbance are included in the measurement, a higher concentration is usually ascribed to the test material. More modern methods for the chemical analysis of secondary chemical constituent markers in botanical products involve some form of chromatography. Thin-layer chromatographic procedures have the advantage of being simple, rapid, can provide useful characteristic profile patterns, and are inexpensive to use. However, their resolving power is limited and quantitative date for minor constituents are difficult to obtain. Gas chromatography (GC) can provide high resolution of the more volatile complex mixtures, but is of limited value in the case of non-volatile polar compounds, especially the polar polyhydroxylated and glycosidic compounds. High-performance liquid chromatography (HPLC) is capable of resolving complex mixtures of polar and non-polar compounds, and has become the chromatographic method of choice for the qualitative and quantitative analysis of botanical extracts and products. HPLC can be coupled with a range of analytical detection techniques including ultra-violet (UV) spectroscopy, evaporative light-scattering detection (ELSD), and mass spectrometry (MS) to produce a "fingerprint" of the botanical product, and/or to quantify the concentration of one or more of the active markers. Detection by UV is readily available in most labs, and is carried out either with a single- or dual-wavelength, or a full spectrum (e.g. photo-diode array) detector, and is the most appropriate technique for the routine analysis of compounds that contain a UV-active chromophore. Combined HPLC-mass spectrometry (LC-MS) and liquid chromato-graphy-tandem mass spectrometry (LC-MS^n) is being increasingly used. The advantage of these methods is that as each compound elutes, the mass can be determined by the mass spectrometer, elemental composition can be determined using exact mass measurement, and structurally significant fragment can be detected, which can provide specific identification of the eluting "peak". A third commonly used detection technique is evaporative light scattering detection (ELSD), as a vast majority of all plant secondary metabolites are detectable whether or not they contain a spectroscopically active chromophore.

In the case of the two herbs under study for safety and efficacy, the bioactive marker compounds in T. pratense were identified as the iso-flavones formononetin (1), daidzein (2), biochanin A (3) and genistein (4). These compounds possess conjugated aromatic chromophores, which are amenable to HPLC-UV detection, and were so analysed (Booth et al., 2006). The bioactive markers actein (5), 26-deoxyactein (6) and 23-epi-26-deoxyactein (7) identified in C. racemosa, on the other hand, contain no UV absorbing chromophores, and hence, cannot be analysed by HPLC-UV. This problem was overcome by the use of HPLC-ELSD detection (Fabricant, 2006).

Once prototype standardized extracts were formulated, one of our industrial partners, Pureworld Botanicals, South Hackensack, NJ, a GMP certified manufacturer of botanical extracts prepared the clinical extracts per our specifications for subsequent pre-clinical and clinical studies.

Production of biologically and chemically standardized dosage forms

In the safety and efficacy evaluation of herbal supplements/medicines, an important consideration is the dosage formulation, which can affect the bioavailability of the active chemical constituents present in the standardized clinical extract. For oral administration, one may consider the use of liquid (syrup), hard capsules (gelatin), soft gel capsules, tablets, or even powders. Each of these formulations has its unique characteristics, advantages and disadvantages. Liquid and powdered dried extract dosage forms can facilitate absorption, but the production of a placebo with the same taste and smell is most difficult. For non-polar constituents,

soft gels containing an oil-based vehicle, offer greater degrees of absorption, however, this may be problematic in the case of polar compounds. Hard shell capsules and tablets are the most popular oral dosage forms. In the case of tablets, the presence of binders and excipients, plus the compacting of the mixture can lead to disintegration and dissolution problems, which in turn lead to questions of bioavailability. As an example, a study on the pharmaceutical quality of melatonin tablets by Hahm *et al.* (1999), three of five immediate release tablets (different brands) failed both the USP tablet disintegration (30 min) and dissolution tests, with two of them not disintegrating after more than 20 h. There is an obvious manufacturing problem, perhaps due to excessive compacting of the tablets during production. In our own post marketing study of ginseng products as part of the American Botanical Councils Ginseng Evaluation Program (Hall *et al.,* 2001a,b), we found that the detectable content of ginsenosides was much higher in the hard gelatin capsule (> 3% total ginsenoside) than in the corresponding tablet (< 7%) formulations (Fitzloff *et al.,* 1998). The disparate quantifiable ginsenosides in the two formulations was probably due to the fact that in the case of the tablets, the analytes were much more tightly bound to the binders and excipients in the tablets than in the capsules, and hence might have been incompletely extracted.

Given the Hahm et al. report and our own ginseng evaluation experience, we concluded that the use of opaque hard gelatin capsules would be the best dosage formulation for our clinical studies. Having made this choice, we then had to decide on what excipient material to be used to formulate our clinical extract. For many years, lactose has been the most commonly used excipients for gelatin capsules. However, it has been shown in recent years there are a number of individuals in the general population, who are lactose-intolerant. To avoid such potential problems in our study subjects, we elected to use rice powder as the excipient for our clinical dosage form.

For our clinical studies, the test materials and their corresponding placebo and positive control capsules were prepared under GMP conditions by Pharmavite, Mission Hills, CA and our other industrial partner.

Pharmacokinetics and bioavailability

It is generally recognized that unlike single ingredient pharmaceuticals, pharmacokinetics and bioavailability studies of herbal medicine, especially poly-prescriptions, containing a multiplicity of chemical constituents, many of which are unknown and/or unidentifiable, are very difficult, if not impossible, to conduct. If one of more bioactive constituents are known, limited studies involving these compounds can be conducted to provide some information. In a limited pharmacokinetics study on T. pratense, we encountered no bioavailability problems as demonstrated by our being able to quantitatively determine the level of the bioactive markers formononetin (1), daidzein (2), biochanin A (3) and genistein (4) in the blood and urine samples of five health women subjects in our phase I study (Piersen *et al.,* 2004).

CONCLUSION

Evidence for the efficacy and safety of herbal medicine must be established by scientifically valid research, the conduct of which is filled with challenges ranging from the pre-clinical determination of the quality of the source material; chemical and biological standardization parameters and methodologies; choice of clinical formulations and GMP manufacturing; to the clinical evaluation of the formulated products. Herbal-derived remedies need a powerful and deep assessment of their pharmacological qualities and safety issues due to the large and growing use of natural-derived substances all over the world, which cannot rely only on the tradition or supposed millenarian beliefs; explanatory and pragmatic studies are

useful and complementary in the acquisition of reliable data both for health caregiver and patients.

Evidence-based medicine(EBM) was first conceived by Archibald Cochrane as a cultural and methodological approach to clinical practice to make decisions; based on clinical expertise and the most intimate knowledge of the individual patient's clinical situations, it de-emphasizes unsystematic clinical experience as ground for medical decision-making and stresses the rigorous analysis of evidence from clinical research. An important problematic of EBM is the difficulty to be easily applied in everyday practice, in a ABC system, especially in the field of traditional medicine and probably pragmatic studies can be an useful tool in reaching this major objective as part of the systematic process of knowledge.

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