

CLINICAL TRIALS IN PHYTOTHERAPY: FROM CONCEPT TO PUBLICATION

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INTRODUCTION

In the early 1990s, after many years as a teacher and researcher in nutrition at The University of Reading, I retrained as a phytotherapist. This new training has influenced my research direction, which is now focused on two areas: hormone imbalance in women (e.g. premenstrual symptomology) and type II diabetes, using a nutritional approach and, increasingly, phytotherapy. I shall illustrate this talk with examples of my own research in both subject areas, highlighting problems and solutions in conducting human studies in phytotherapy.

Research in phytotherapy is in its infancy - particularly here in the UK - and can be informed from techniques developed in the field of nutrition over recent years. Nutrition is also a young science and there are many parallels between the two subject areas. Indeed, in some ways phytotherapy can be thought of as a branch of nutrition, since phytochemicals, - the active components of herbs - are now well accepted as being nutrient accessories and even termed “non- essential nutrients” in a UK Government document (DoH, 1994).

Much of the growing research interest in phytochemicals stems from the increased understanding on the part of nutritional scientists that, as well as the accepted ‘essentiality’ of nutrients (adequate nutrition to avoid deficiency disease), nutrient ‘optimality’ is a valid target concept (adequate nutrition to avoid chronic disease and protect from environmental toxins). The accumulating evidence for a positive role in health maintenance and disease prevention of antioxidants has fueled this rather abrupt change in thinking. Protective antioxidants are now well recognised to go beyond vitamins to include the phytochemicals such as flavonoids,

carotenoids, coumarins etc, many of which demonstrate even greater antioxidant activity than nutrients. Fruit and vegetables are good sources of these substances and herbal medicines are even richer sources. Medicinal effects are ascribed to phytochemicals, whether from plant food (Walker, 1996) or non-food (herb) sources.

Being conversant with both nutrition and phytotherapy allows me to draw useful parallels between the two subjects areas which may be valuable in identifying research strategies for phytotherapy.

COMPARISON OF THE DEVELOPMENT OF PHYTOTHERAPY WITH NUTRITION

History of Nutritional Science. Historically, the science of nutrition has progressed through several stages, which have culminated in a realisation of the merits of a holistic approach to diet.

Over the centuries, clinical experience led first to foods being identified which were able to cure nutrient deficiency disease. Examples are the use of whole rice to cure beri-beri (now known to be thiamin deficiency) and citrus fruits to cure scurvy (now known to be vitamin C deficiency). The next step early this century was the identification of individual nutrients through the increasing sophistication of analytical techniques (Walker, 1997). This process ended in the late 1940s with the discovery of the last vitamin - vitamin B12.

From the 1980s onwards, nutrition research has taken another direction, to reveal clear evidence that the benefits of a healthy diet go beyond the sum of the effects of single nutrient components. This

knowledge has come about through improvements in epidemiological techniques and human experimentation. Indeed, we are now so confident of the protective effects of fruit and vegetables against cancer that we are in a position to give advice to the public on their role in disease prevention (Block et al., 1992; DoH, 1998). Cancer is the most feared of the chronic diseases, but others which are prevalent at the end of the 20th century, such as heart disease and diabetes, contribute to premature death and long-term poor quality of life. The incidence of these too can be reduced with diets high in plant foods (DoH, 1994).

The particular components of fruit and vegetables which contribute to reduction of disease risk are still a matter for debate, but increasingly it appears that the merits of this food group go beyond their essential nutrient content and involve the myriad of phytonutrients present. Little is currently known about the physiological effects, let alone the mechanisms of action, of these phytonutrients, so there is a long way to go before research is sufficiently advanced to provide a clear explanation of the mechanisms behind the merits of the whole diet approach to health. In the meantime, we have come full circle in our approach to nutrition: we know enough about efficacy from research studies on humans to give the public health messages on whole diet which will have a real impact on their health.

History of the Science of Phytotherapy

The development of the science of phytotherapy shows many parallels with that described for nutrition. The traditional use of mixtures of herbs dates from the earliest written records from several, previously isolated, cultures spread throughout the world. However, it was only from the 1950s onwards that analytical techniques were sufficiently advanced to allow the identification of key active constituents in individual herbs. Now most herbal medicines have been adequately defined from a chemical point of view to account for their clinically observed actions.

From the 1980s onwards human studies were undertaken, particularly in Germany on standardised extracts of single herbs. Although positive demonstrations of efficacy in double-blind clinical trials showed the potential therapeutic potential of these herbs, this approach ignored the possibility of synergy between herbs (termed polypharmacy) as used by phytotherapists in prescriptions. Clinical experience of synergy is common knowledge to phytotherapists, but it will be a big challenge to demonstrate it. Nevertheless, I am confident that the positive demonstration of synergy only awaits the will, the funds and the appropriate protocols to provide data to support the phytotherapists' contention of the value of herbal combinations.

Documentation of traditional use

The criteria required by regulatory bodies for a plant food for use as a nutrient compared with the same food used as a medicine differ. As a food, evidence of tradition of use is required, but not necessarily needed in a documented form: anecdotal evidence may suffice. All the foods that we eat today we only consume because their safe use has been verified by our ancestors. A tradition of food use means that there is no requirement for toxicological testing or proof of efficacy of the food as a source of nutrients, according to most systems of legislation throughout the world. A good example is the GRAS (Generally Recognised As Safe) List of the United States, which is a list of natural ingredients with tradition of use status which are exempt from toxicological testing requirements.

Clearly, the provision of documented evidence of use becomes more important for herbal medicine if the plant has not been used as a food. Unfortunately, collation of documented evidence of tradition of use, including modern use, is much underrated as evidence for the continued human use of herbal medicine for regulatory purposes. There is a need for the help of enthusiast historians, who are prepared to delve into the old herbals across various cultures, to collate this information for each herb in the materia medica.

Clinical experience of efficacy of phytotherapy

Documenting modern use of herbs by medical herbalists also provides valuable resources for the future. We conducted a survey of members of the National Institute of Medical Herbalists (UK) (Christie & Walker, 1998). This survey focused on the use of Chaste tree berries (*Vitex agnus-castus*) for women's health problems, particularly in treatment of adverse premenstrual and menopausal symptoms. The data obtained provided an opportunity of comparing modern and traditional use. As well as yielding valuable information on the therapeutic profile of the herb, perception of efficacy, form of preparation and dosage used, the use of *Vitex* was compared with 12 other specified herbs, of which the seven most commonly used can be seen in Table 1. No doubt a phytotherapist would have predicted the responses obtained and therefore have regarded the exercise as futile. However, documenting these responses is valuable in making this knowledge available for posterity as supporting evidence of the traditional and current use in practice of *Vitex*.

Although, as for foods, no toxicological testing of medicinal plants is required by law, toxicological data exists for most herbs in the form of acute toxicity data (e.g. LD50) collected unsystematically in academic institutions. Setting aside those herbs high in toxic alkaloids, the data for the vast majority of herbal medicines indicate low toxicity. Clearly, it is for efficacy rather than for safety where more data is needed for phyto-medicines. Proof of efficacy using human subjects in clinical trials as described in the remainder of this paper is increasingly demanded by regulatory agencies.

Research tools available for phytotherapy

(a) Observation. Figure 1 gives an overview of the observational and experimental research methods available for phytotherapy compared with nutritional science. Observations of progress of treat-

ment of individual patients is a method open to both nutrition and phytotherapy which is useful in the clinical context, but is of limited value in drawing general conclusions regarding efficacy.

Improvements in methodology of epidemiology have greatly progressed the identification of possible relationships between diet and disease in the field of nutrition. Although epidemiology is not available to the phytotherapist (herbal medicine is not consumed on a regular basis by the general population), the collation of practitioner records would serve well instead. Unfortunately, there has been little published data of this type. Indeed, there is a desperate for need to develop research methodologies for following practitioner experience of treatment. Both retrospective and prospective methodologies need to be developed to yield publishable data of value to a wide audience.

(b) Experiment. In vitro techniques and animal studies are available to the phytotherapist as they are to the nutritionist for the testing of safety and efficacy of natural products. In vitro methods such as cell culture are useful in establishing mechanism but give little information on efficacy. Researchers in phytotherapy can gain from the experience of those working in the field of nutrition. This has taught us that the discovery of fancy mechanisms from in vitro and/or animal experiments, which are not backed by epidemiological evidence, fail to progress our knowledge of human nutrition. For this reason, but also for ethical reasons, less importance is placed on animal data these days.

For proof of efficacy, the clinical trial has become of paramount importance in nutrition, so much so, that, unless a study has been conducted with human subjects, no pertinent conclusions can be drawn. A similar scenario is developing for phytotherapy and this has been given impetus by demands of regulatory agencies for proof of evidence of phyto-medicine in human studies.

STAGES OF A CLINICAL TRIAL

Many people who have not been involved in clinical trials are unaware of the wide range of skills needed to successfully proceed from concept to publication. These stages are shown in Table 2. To develop a workable protocol, to get it allowed by an ethics committee, to successfully recruit volunteers within inclusion criteria, to execute the study and to collect and analyse the data requires focus, tenacity and organisational skills as well as aptitude for written and oral presentation.

The trial concept needs FOCUS

Formulation of the research question to be answered is very important before beginning the design of the study. This question should be recorded and discussed with colleagues at an early stage in the planning of any study. In phytotherapy it may be that the researcher will choose a disease condition to be studied and ONE of the following:- (a) the herb, (b) the herbal medicine, i.e. a traditional mixture of herbs (polypharmacy) or (c) the effect of the practitioner. Although there are many questions which may need answering, introducing too many in one study risks a failed experiment. Fortunately, ethics committees are on the lookout for human experiments that are likely to fail and advise accordingly, because it is not ethical to waste volunteers' time in a study unlikely to succeed.

Study Design

It is important to define clearly the target population to be studied. Hence, depending on the exact nature of the study, it may be necessary to consider exclusion or inclusion on grounds of gender, age, medication, disease risk factor biomarkers, medical history, stage of disease, diet, anthropological measurements etc.

Strategic considerations in designing a clinical trial are shown in Table 3. Should it be open and uncontrolled? Clearly, it would be preferable if a study

were placebo controlled as a successful study with double blinding will be easier to publish in a high quality journal and hence the results will be available to a wider audience. Other points are amplified below.

The placebo: ethics of use

Several phytotherapists have expressed their concern about the use of placebo for disease conditions. In my view their concern is entirely justified in terminal conditions and for progressive conditions, such as type II diabetes. However, for more minor ailments, such as premenstrual symptoms, I cannot see why there should be objection to the use of placebo, as the placebo effect itself can be considerable and contribute greatly to improved well-being, as we have shown in a study of magnesium supplementation for this condition (Walker et al., 1998).

Even in a study of type II diabetes, there are ways around the ethical problem of placebo by ensuring all patients continue to receive some treatment. In a study we undertook on 26 type II diabetics (Marakis, 1998) volunteers continued on their normal medication and diet and we randomized them to receive, as adjunct therapy, either a multivitamin and mineral supplement or a placebo. There may be scope for phyto-medicine to be used as adjunct therapy in a similar way, as, generally, the concurrent use of phytotherapy with orthodox drug treatment is not contraindicated.

We used another approach in dealing with the ethics of placebo use in a study in Uganda (Kikafunda et al., 1998). We set out to investigate the effect of zinc supplementation on growth of preschool children, but the use of placebo for malnourished children raised a serious moral issue. However, after taking advice from a pediatrician with experience of human studies overseas, we agreed on the compromise of dissolving both the zinc and placebo in freshly-prepared fruit juice, which was administered to the children on each school day. Hence all the children received some nutritional benefit in terms

of extra nutrients, such as vitamin C and folate, by participating in the study. Our data showed an improvement compared with placebo in mid-upper arm circumference of all children in the zinc-supplemented group and in the growth of the children from the more well-to-do of the three schools studied, on zinc supplementation. These results led us to propose that in poorer situations, other nutrient deficiencies preclude the full benefit of zinc supplementation.

Choice of placebo

If a placebo is to be used, then the choice of the placebo is important. The use of olive oil as a placebo in studies of the effects of polyunsaturated fatty acids on biomarkers of heart disease risk, as was common in the past, is now considered unsatisfactory since monounsaturated fats have been shown to influence blood lipid levels.

Our randomized study of the dose-response of the effect of a daily supplement of magnesium for two months on premenstrual symptoms in 85 women revealed a surprise finding! The placebo had a significantly greater effect in reducing overall symptoms, including symptoms of anxiety, than the highest dose of magnesium. At the same time the placebo showed a significant reduction in output of magnesium in the urine compared with baseline. This news was received with dismay by my research student, but I reminded her of the advice of the great nutritionist, Dr Elsie Widdowson in her “Advice to a young scientist” in which she said “Treasure your exceptions” (Ashwell, 1993). Our results clearly showed the placebo had exerted an influence on magnesium homeostasis in women suffering premenstrual symptoms. We searched the literature for explanations and developed a hypothesis which we have now prepared as a paper for the journal *Medical Hypothesis* (De Souza et al., in preparation).

Choice of herbal preparations for clinical trials

Extracts of herbs generally take two main forms, liquid or solid. Phytotherapists in the UK favour

the former as herbal tinctures or fluid extracts (extracted in aqueous alcohol), because these can be conveniently mixed according to traditional practice. Other fluid forms include infusions, decoctions, syrups and glycerol extracts. Solid crude extracts are dried or semi-dried aqueous alcoholic extracts with or without standardisation of one or more key active constituents. Although it is not impossible to design a protocol for use of the fluid extracts, it is more difficult to blind treatment against placebo because of taste and other sensory characteristics. Tablets or capsules are much easier to incorporate into a research protocol, although their use may not always answer the study concept.

It is clear that currently the most important aspect of phytotherapy research requiring attention is proof of efficacy of herbs using human subjects, but at the same time we should also be mindful of mechanism(s), despite my earlier comments. Likely multiple mechanisms should be investigated using biomarker outcomes wherever possible, to advance the science of phytotherapy. Hence, while focusing on the importance of efficacy as primary outcome, secondary outcomes of biomarkers to indicate mechanisms are much more likely to profit our understanding of phytotherapy than searching for mechanisms in isolated *in vitro* experiments. From this point of view, the more that is known about a herbal extract in a clinical trial, the better. Standardisation of a herbal extract is a quality control measure yielding limited information, as it is normally aimed at ensuring a minimum amount of a single active constituent in a crude (traditional) extract. However, multiple constituent standardisation is becoming available for the future. In the meantime, it would be sensible for researchers in phytotherapy to freeze samples of experimental herbal materials used in trials for subsequent analysis.

Estimating volunteer numbers

It is becoming increasingly important to ethics committees and for the acceptance of scientific papers by journal editors to show that a reasoned

argument has been made in estimating volunteer numbers for clinical trials, so that the chances of a successful outcome are maximised. This can be done by finding data from a similar study to predict the variability (e.g. standard deviation) of the primary outcome chosen. If such a study does not exist, then a pilot study should be undertaken. It is essential at this point to consult a statistician and to agree on a meaningful change in primary outcome compared with placebo. Once variability and expected change have been agreed, then the number of volunteers required can be calculated to give a specified significance level with a known power (e.g. significance at the 5% level with 90% power). Allowance will need to be made to accommodate dropout.

Figure 2 shows the results of a pilot study which we conducted on the effect on resting diastolic blood pressure of a daily supplement of 600 mg Mg and/or 500 mg standardised extract of Hawthorn (*Crataegus oxyacantha*) on 38 mildly hypertensive subjects (Marakis, 1998). This was a double-blind, placebo-controlled, parallel study with about 10 people in each group. As the numbers were small, analysis of variance (ANOVA) did not show significant difference of any single treatment group compared with placebo. However, extension of the ANOVA to factorial contrasts (effect of magnesium or the effect of Hawthorn) showed Hawthorn extract to have a non-significant trend ($P = 0.081$) towards lowering blood pressure.

One problem of our pilot study was that the means of the baseline diastolic blood pressure of the groups were significantly different and this lowered the power of the study. Nevertheless, the trend observed in this pilot study indicated that a larger study should now be undertaken. Also, data from the pilot study can now be used in a power calculation to predict volunteer numbers likely to lead to a successful outcome in a larger study. Our pilot study also demonstrated that the protocol for a larger study should ensure that randomization of volunteers is stratified to achieve groups without baseline differences in

blood pressure, as this will increase the power of the trial.

Choosing outcome

There is a danger in any study where there are several outcomes measured at various stages, of applying multiple statistical testing and finding a significant difference between treatment and placebo by chance. This is because, from a statistical point of view, for any 20 tests conducted, one may be significant by chance at the 5% significance level. Hence it is important to identify primary outcomes in the protocol of any study. Possible outcomes of studies into phytotherapy are shown in Table 4.

Despite the dangers of multiple testing, if an outcome is easy to measure it should be included as a secondary outcome, but it must be made clear in publication that it had this status in the protocol. In a study of the effect of a daily vitamin and mineral supplement for three months on glucose control and blood lipids of 26 type II diabetics, secondary outcomes were the responses to a validated well-being questionnaire. The study was a randomized, double-blind, placebo-controlled crossover study with one month washout between the study arms. The results of this questionnaire turned out to be highly significant - more so than the main outcome (Marakis, 1998). There was a significant drop in anxiety and a significant increase in vitality whilst on the supplement compared with placebo. Armed with this knowledge, in our next study planned for 1999 on the effect of two herbal extracts on type II diabetes, we plan to include the results of the same questionnaire as primary outcome.

Ethical clearance

Ethical clearance can only be sought once all the factors above have been considered. Then, it is important to gain the confidence of the members of the ethics committee with a well thought out application. A proactive attitude is helpful, particularly as members of the committee may not be familiar

with phytotherapy. Clearly, a fully referenced protocol comprising background, aims, study design, outcome(s), cooperation with outside bodies, statistical treatment of data etc needs to be provided. It should contain, as appropriate, specimens of the volunteer information sheet, volunteer consent form, GP (general practitioner) information letter, GP acknowledgement form, questionnaire(s) etc. However, in addition, for a study of phytotherapy, evidence of use and restrictions of use of herbs and dosages along with appropriate monographs may speed ethical clearance.

Recruitment and study execution

It is important to allow plenty of time to achieve successful recruitment in clinical trials. In our experience, recruiting through the health services (hospital consultants, GP surgeries, clinics) is likely to be less successful than open advertising, as professional people have other priorities. Open advertising may be via posters, local news bulletins, local radio and press, national newspapers or monthly magazines. Patient support groups may also be helpful. It is important that someone is assigned to telephone duty to deal with initial contact to avoid loss of potential volunteers.

Clinical trials require a wide range of skills to successfully proceed from concept to publication, in particular: focus, tenacity, organisation and oral and written communication skills. It is important to think ahead and to pre-empt volunteer queries. Frequent contact, including briefing and debriefing meetings is essential for maintaining volunteer motivation to stay in the study.

Publication in peer-reviewed journal

In publishing research findings in phytotherapy the journal needs to be chosen carefully, since the subject area is alien to many editors. In a very critical examination of the peer review process Horrobin (1990) said

“Peer review must aim to facilitate the introduction

into medicine of improved ways of curing, relieving, and comforting patients. This requires both quality control and the encouragement of innovation.”

He points out that, unfortunately, in striving for quality, innovation is often suppressed because it does not fit into preconceived views. He also warns of vested interest on the part of reviewers, whose own research relies on perpetuating conventional wisdom. Those of us in phytotherapy must be aware that our traditional approaches may be regarded as ‘innovative’, despite their ancient origins and therefore may be treated with scepticism. Horrobin gives examples of suppressed innovation, including Krebs’ article on the citric acid cycle, which was rejected by the peer review process on first submission to a journal!

Hence, although it is hard on the authors to receive news of the rejection of their paper, it is important for them to take a philosophical attitude to manuscript rejection and be tenacious. A paper of ours on the effect of a daily magnesium supplement (200 mg) for two months on premenstrual symptoms in thirty-eight women (a randomized, double-blind, placebo-controlled, crossover study) was rejected twice (European Journal of Clinical Nutrition and British Journal of Obstetrics and Gynaecology) on the grounds of “... no interest to our readers.” and “...other priorities.”. Finally, it was accepted by the Journal of Women’s Health (USA) with “...a great paper” being one comment from the referees!

CONCLUSIONS

As has already occurred in the field of nutritional science, evidence of efficacy of herbal medicine will greatly strengthen the credibility of phytotherapy in the eyes of both the public and health professionals.

Undertaking clinical trials of phytotherapy is demanding and requires the application of many different skills from concept to publication. It is important to be tenacious throughout and particularly

at publication. Good work often fails at the last hurdle, but a study unpublished is of no greater value to posterity than one which has not been carried out.

Although the clinical trial might be regarded as the 'gold standard' for testing efficacy of phytotherapy, other approaches (collation of clinical records and practitioners' experience) are much in need of development as research tools.

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