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Methods in ethnopharmacology

Donald P. Waller

Professor of Pharmacology and Toxicology, Program for Collaborative Research in Pharmaceutical Sciences and Department of Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, Chicago, IL (USA)

A variety of pharmacologieal models are utilized in the evaluation of ethnomedieine. Most investigations are focused on developing new leads for therapeutic agents. However, there should be more efforts focused on the development of ethnomedicines because of their aecessibility and acceptability in areas where modern medieine is not readily available. Testing methods to identify the aetive agents must be carefully selected utilizing information from ethnoanthropology, ethnobotany, phytochemistry, toxicology and pharmacology. New pharmacologieal models focused on cellular and molecular mechanisms can be used for ethnomedical evaluations but with great eaution sinee they are based on known mechanisms of aetions and limited by knowledge of the disease state.

Key words: ethnopharmacology; pharmacology; testing

Introduction

Ethnomedical treatments are used to treat most disease states and medical conditions known to modern medicine. Ethnopharmacological evaluation of the efficacy for these ethnomedical treatments rely upon a large number of different pharmacological models. A discussion of the models in use would require model evaluations for all the major organ systems including the nervous system (central, peripheral and autonomic), the cardiovascular system (glycosides, antiarrhythmic and antihypertensive activities), renal system, reproductive system (male and female), hormone system (thyroid, pancreas, etc.) and a variety of infectious disease models for viral, fungal, parasitic and bacterial infections. Many pharmacological models are also defined in terms of disease states such as inflammation and cancer. Discussions of all these areas are beyond the scope of this manuscript. More reasonable questions on the current status of methods in ethnopharmacology would be to ask:

(1) What are the primary goals of ethnopharmacological investigations?
(2) What is a good scientific approach to ethnopharmacological investigations?
(3) What are the current trends in pharmacological methods?

Correspondence to: Donald P. Waller, Ph.D., Professor of Pharmacology and Toxieology, Program for Collaborative Research in Pharmaceutical Scienees and Department of Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA.

What are the primary goals of ethnopharmacological investigations?

The primary goals of ethnopharmacological investigations are to evaluate and verify pharmacological activity of an ethnomedical treatment. This information can be used to identify efficacious ethnomedical preparations and provide a source of new lead compounds for drug development.

Most of the ethnomedical literature is used as an information source for discovering new active leads for therapeutic agents. The design of pharmacological models also appears to focus on the isolation and identification of chemical entities for this purpose. However, how does this assist the cultures from which these new leads come? The active ethnomedicines usually return in a form not recognized by the original users and too expensive for them to afford. An example would be the new antimalarial arteether, derived from artemisinin, the active ingredient of a Chinese traditional antimalarial medicine for over 2000 years. Arteether is very effective against some resistant strains of malaria but the cost is 10 times greater than chloroquine, making it too expensive for most areas plagued by malaria.

The verification of an ethnomedical treatment for efficacy and encouraging its use as a remedy is an important goal often forgotten or neglected by

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modern scientists. Clearly, some ethnomedical preparations are simple placebos, while others are without any actions and used to treat self-limiting diseases such as minor influenza or a simple cold. However, identifying and characterizing the efficacious ethnomedical treatments for a variety of significant disease states could greatly benefit indigenous populations in several ways and more efforts should be focused on these goals.

Large numbers of people in developing countries have poor access to modern health care systems and the associated drugs used in the treatment of disease. Most countries with frequent usage of ethnomedical treatments have many traditional practitioners preparing ethnomedicines or providing preparation instructions to local populations. These practitioners could be used to great advantage if they were organized and encouraged to use only efficacious · and safe ethnomedicines while discouraging the use of ineffective and potentially toxic ethnomedicines. Such activities could be supported by performing scientific evaluations of efficacy for local ethnomedical preparations as well as organizing and disseminating scientific information to the local traditional practitioners. Limited availability of modern drugs is primarily due to the cost of modern drugs to already impoverished governments. The components used in ethnomedicine, however, are relatively inexpensive and accessible since most ethnomedicines are formulated from locally grown and produced plant products. Most importantly, the cost is usually appropriate for the economic situation of the local area. The availability of a local pharmacopoeia supported by scientific data could positively impact the health status of large populations without access to modern drugs.

The acceptance of ethnomedical preparations by indigenous populations not familiar with modern medicines can be a problem. Capsules and tablets are usually without taste, whereas most ethnomedical remedies have a characteristic odor, taste and texture which accompany their administration. The power of these aromas, tastes and textures can be as important for compliance as the active ingredients which may be contained within the preparations. Indeed, it is difficult to convince a person who has never encountered Western medicine of the activity of a small white tablet. The storage and the repeated administration of a tablet may also be difficult when local climactic and living conditions make it impossible to store the dosage form under appropriate conditions. The storage of herbs and locally grown materials, however, may be easily described and understood by local populations or traditional practitioners.

The use of an ethnomedical treatment may in some cases be more appropriate than modern day remedies. Diarrhea causes millions of deaths in children less than 5 years of age. Tetracycline and diphenoxylate hydrochloride can be effective prophylaetic or acute treatments in many cases and are often used by travellers. Such treatment regimes however, would not be appropriate for repeated insults from a variety of unknown etiologies which typically occur in many indigenous populations. The drugs are very expensive for developing countries and long term repeated use of such drugs could result in the development of resistant organisms, superinfections, hypersensitivities and abuse. A mild remedy for treating such problems as diarrhea may certainly be both more effective and more appropriate. Oral rehydration therapy (ORT) using trisodium citrate dihydrate/sodium bicarbonate glucose, and cooked rice powder, is available for the treatment of severe diarrhea in many areas where people have access to modern medicine. However, this simple treatment is still not readily available to many people who suffer the most.

There are many ethnomedical preparations used to treat diarrhea. Dissemination of information for an effective ethnomedical preparation could lead to its widespread use. Appropriate plants may be identified in a local area, such as the guava plant (*Psidum guajava*) which is easy to grow and readily available in most areas. Chewing the leaf or use of the rootbark of the guava plant as a decoction has reported antibiotic, antiinflammatory and antidiarrheal activities. Such activities may be due to the presence of one or several components; however, there is no reason to determine the active ingredient(s) if the preparation is efficacious and used in its natural state. Clinical trials to evaluate an antidiarrheal would be difficult. The questions of monitoring, etiology of the disease and the selection of patient populations are certainly not easy to answer. In addition, the quercitin present in this preparation raises some concern for potential long term adverse effects. However, considering the potential benefits, such problems must not prevent an effort directed towards identifying and encouraging the use of active ethnomedical preparations for disease states such as diarrhea, which afflict large populations of people.

The scientific community ignores the potential of identifying appropriate efficacious ethnomedi-

cal preparations and encouraging their use. Ethnomedical preparations would certainly not meet the good manufacturing standards of modern drug production or provide profits to drug companies, but they could provide large populations better access to efficacious drug treatment and an improved health status where modern health practices have failed or are not available.

What is a good scientific approach to verify ethnopharmacological activity?

A good scientific approach to ethnopharmacological investigations requires the use of information from several disciplines. Ethnoanthropological, ethnobotanical, phytochemical and toxicological information must be obtained and evaluated along with the ethnopharmacological data.

A rational choice of an appropriate pharmacological method to verify ethnomedical activity depends upon an accurate determination of the disease state or condition for which the preparation is used. During the past year I have reviewed more than 100 manuscripts submitted to the *Journal of Ethnopharmacology*. The pharmacological evaluations are often not adequately justified using ethnomedical information. The ethnomedical information is either poorly associated or absent in the manuscripts. The selection of a pharmacological method to evaluate an activity, without good ethnopharmacological justification, is the same as randomly screening plant materials for pharmacological activity. Complete ethnomedical information is essential. A challenge must go to the ethnoanthropologists to collect and/or properly interpret information to associate a particular remedy with a relatively well defined disease state. Some diseases are somewhat simple to diagnose from symptoms whereas others may be impossible to diagnose without modern day laboratory evaluations. When the disease state being treated has relatively distinct symptoms and there is long term experience with the disease, the ethnomedical treatments can be associated with a disease condition. An appropriate model for the disease state can then be selected to verify activity, assuming there is an appropriate model for the disease state.

Malaria is reasonably well characterized and indentifiable. Experienced health practitioners, modern or traditional, can usually recognize the symptoms of malaria with a reasonable degree of certainty without laboratory testing. Some aspects are more difficult to assess, such as the identification of the different types of malaria organisms. However, even this may be determined with careful observations of the onset and duration of fever and the period of time between episodic attacks which characterize the different malaria organisms. Relating the efficacy of an ethnomedical preparation to a type of malaria which may have known resistance to current antimalarials may be possible with complete information on the symptoms of patients being treated.

Imprecise terms are often found in the ethnomedical literature. Inflammation as a diagnosis presents a most difficult situation. Inflammation can occur in most organs. Some examples are the lungs, the eyes and the skin. The causes could be trauma, infection, allergy or asthma. The literature has many references to the antiinflammatory activity of various ethnopharmacological treatments with little or no supporting information on etiology. Treatment of inflammation could be through antibiotic, antihistaminic, analgesic or other specific mechanisms which mediate swelling and inflammation. Modern pharmacological agents used to treat inflammation indeed represent a broad spectrum of activities including corticosteroids, antihistamines, epinephrine, non-steroidal antiinflammatories and antimicrobials. Thus the simple term of antiinflammatory activity does not provide adequate information to select a model for pharmacological evaluation. A more detailed description of the disease state would be required in order to determine what mechanisms caused the inflammation and what testing would be appropriate for a particular ethnomedical preparation.

Appropriate identification of the plants in ethnomedical preparations evaluated in pharmacologic models is an absolute requirement. Plant identification and evaluation of the botanical literature for nomenclature prevents unnecessary duplication of work or confusion due to synonyms. The ability of a pharmacologist to repeat an experiment involving a plant product may require repeated collections at a later date by other persons. When, where, how and conditions of the collection are essential. Location and the time of year can have a significant impact on the constituent levels present in many plants. Some pharmacologists fear if the botanist provides the sample and all the detailed information, they will also insist on having their name on the resulting manuscript. It is appropriate to have a botanist on a pharmacological publication. In fact, it sends a

message that a multidisciplinary approach was used in the study, assures proper identification of the plant, and reassures the scientific world that the experiments performed could be repeated.

A comprehensive information base on phytochemical constituents also is essential prior to the onset of pharmacological testing. Active compounds may have already been identified in a plant. It is a surprise to many pharmacologists that different plants, even in unrelated species, may have common active consituents. Some compounds may also interfere with a testing method. Tannins, antioxidants, amino acids and particular saponins are all structures known to interfere nonspecifically in pharmacological testing and must be considered when selecting a pharmacological model. Toxic constituents must also be identificd prior to method selection. Toxicants in an ethnomedical preparation may often cause a positive pharmacological effect through toxicity or be too toxic to be useful. There are several examples. The Vinca alkaloids, active anticancer agents, would be active in a reproductive screen, but surely would not be good candidates as antifertility agents. Pyrrolizidine alkaloids are present in a number of plants. These potent liver toxins are also active in antifertility screens due to their effects on fetal liver but are too toxic to be considered as an antifertility agent. There are good resources for accessing phytochemical constituent information such as the NAPRALERT database available in our institution.

Dosage forms of ethnomedical preparations take many forms. Decoctions, poultices, inhalants and powders represent a few of the more common forms. A clinical trial would probably use the ethnomedical dosage form. However, most validation testing procedures use animal models or some type of in vitro screen where the original dosage form would not be appropriate. Most decoctions, for example, have volumes not easily adapted to an animal model. This makes extraction and concentration of the preparations a necessity. Pharmacologists like working with pure, water soluble, single receptor site agents which produce sigmoidal curves during pharmacological testing. What the chemist usually provides is a complex, water insoluble, gunk or tar. Often the most active components are found in the most water insoluble fractions, whereas most biological systems utilize an aqueous matrix for pharmacological testing. This results in a very difficult problem related to the solubility of the material to be tested and its bioavailability to the test system receptors or sites. There may be a requirement for solubilization or formation of a coprecipitate to make the test substance accessible to the test system milieu. Improper precautions will certainly result in inactivity of a potentially active compound.

A route of administration must be carefully selected and may be dependent upon the type of product available for testing. Most concentrated extracts have a very irritating nature when administered by a parenteral route of administration. Repeated subcutaneous administration of a nonpolar plant extract will frequently cause a local tissue response which may reduce absorption and result in a false negative response. Certainly, a large area of irritation in the peritoneal cavity will result in severe damage to most abdominal tissues and large changes in physiological function. This route is often used in evaluations of ethnomedical medicines, yet rarely will any mention of the toxic manifestations be found. Intravenous injections of water insoluble or particulate substances are totally inappropriate and often found in the ethnopharmacological literature. It is fairly obvious why such preparations would alter hemodynamic function, whether or not active pharmacological agents were present.

An appropriate pharmacological target will be the primary consideration for the pharmacologist. An obvious model system to validate a reported ethnomedical treatment is man. Clinical trials using ethnomedical preparations can be performed in a number of countries where there is a long history of use for a particular product. Unfortunately clinical trials are difficult, expensive and often considered unnecessary for preparations used for centuries. Indeed, it is even more difficult to convince someone of potential toxicities associated with its consumption. Clinical trials of ethnomedicines in most developed countries are impossible due to the eostly preclinical safety requirements of drug regulatory agencies. It is surprising, yet understandable, that few clinical trials have been published on efficacy testing of ethnomedical preparations. Thus some other means of testing must be utilized to identify active preparations.

Traditionally pharmacologists have used in vivo, in situ or tissue models. These methods were developed and utilized during a time when the understanding of disease mechanisms and the associated pathology was limited. The testing methodologies were often imprecise and varied considerably from laboratory to laboratory in their details. The methods used to investigate 'anti-inflammatory activity' is an example.

Pedal edema in the hind paw of the rat was the

mainstay of such pharmacological investigations. Pedal edema could be induced by a variety of agents including carrageenan, dextran, enzymes, trypsin-hyaluronidase, serum and proteins, antigens including bordetella pertussis, egg albumin, BSA, crystals of calcium pyrophosphate, microbes, and Klebsiella and Streptococcal cell wall fragments. Some dyes, turpentine and gum arabic were also used as inducers of the inflammation process. However, many tissue sites are involved in inflammation. Other tissue models were developed as well to monitor responses. Plasma exudate volume, hyperemia/increased blood flow to tissue, polymorphonuclear leukocyte infiltration, hemorrhage, sponge implantation, injection of irritants and antigens for experimental production of pleurisy and pouch inflammation represented an expansion of the sites and inflammation inducing agents.

Investigators face very complex question. Which model mimics the inflammation being treated by the ethnomedical preparation? What site for insult, what agent to induce the inflammation, and what should be monitored are all part of the decision process. If the preparation is inactive, does that mean the preparation is not active, or merely improper methodology was selected? When would you stop evaluating different models before you are sure that activity was not present? No simple answers to these questions exist. The question of why a particular model did or did not respond usually remains a mystery of the particular agent until an evaluation of the pharmacological activity is completed in many models over many years. The choice of the model system to evaluate an ethnomedicine is a complex and multifacted process. Every effort must be made to select the appropriate model to insure the result will be both accurate and informative.

What are the current trends in methods used in pharmacological investigations?

Scientists in many developed countries are under extreme pressure to reduce or eliminate the use of animals in testing. The science community is responding by applying the advances in knowledge about cellular, enzymatic and molecular mechanisms of disease states and conditions to the development of techniques with reduced or eliminated animal testing. In vivo and animal intensive models are becoming obsolete and their use discouraged. Cells, enzymes and receptors involved in mediating tissue responses are the focus of new biomolecular testing methodologies to obtain drug-effector interaction information. The data from these methods are becoming the basis for decisions on pharmacological activity and toxicity. Most fields of pharmacological study, including cancer, inflammation, reproduction, central nervous system, renal and cardiovascular, are developing new in vitro models.

An in vitro model for a specific pathway can provide important information on drug-effector interactions. The cascade of autacoids responsible for inflammation has only recently been elucidated and adapted to pharmacologic investigations. The arachidonic acid cascade mediating inflammation involves the production of prostaglandins (cyclooxygenase), thromboxanes (15-lipoxygenase) and leukotrienes (5-lipoxygenase). Analytical methods developed to monitor cascade products now provide the basis for monitoring effects on the anti-inflammatory response cascade. The discovery of non-steroidal antiinflammatory drugs (NSAIDs) suppressing cyclooxygenase activity, provided support for using the enzymatic pathways as a screen for antiinflammatory activity.

Such systems have distinct advantages over the older whole animal or tissue models. They utilize fewer animals, can often be performed relatively quickly, and most importantly, reduce costs when testing large numbers of samples. The adoption of these new methods by ethnopharmacologists, however, must be done with some caution.

The cellular or biomolecular approach can only be effective when extensive knowledge is available. The question must be asked, 'How much do we know about the disease condition or process for which we are attempting to establish a drug evaluation model?' An in vitro model determines the effects on the one cell/enzyme/pathway monitored in the test. Most of these models are based on interactions with known pharmacological agents and established mechanisms or pathways. This is in contrast to whole animal or tissue methods which usually evaluate end results, without regard for detailed information on a particular mechanism. It is quite possible, and in fact most likely, many undiscovered mechanisms and pathways exist, which mediate disease, some used by ethnomedicines. It is difficult if not impossible to develop a biomolecular model for an unknown pathway.

Each ethnopharmacologist must determine the best approach for pharmacological testing based on the availability of information, laboratory sophistication and focus. The use of a single in vitro test usually is a minor piece of information adding little to the overall picture of potential ac-

tivity. Fragmented data will be produced when only one or a couple of the in vitro systems are used to generate data. Careful consideration of the types and numbers of assays must be part of the development of a series of tests to evaluate a type of activity. Most in vitro screens require a battery of tests to provide adequate information.

The study of anticancer agents has been the focus of screening programs and can provide an example of the use of a battery of tests to provide broad coverage. Cancer is certainly not one disease, but a collection of similar disease states with characteristics of unregulated cell growth, angiogenesis and metastasis. There are more than several hundred different types of cancers, depending upon how you define cancerous tissues. Events leading to cancer and the mechanisms and the factors in cancer processes such as initiation, metastatic growth and drug resistance are being intensively investigated. This has led to the development of in vitro models which utilize known cancer cell lines and molecular mechanisms involved in cancer as the basis of models. What would be considered a reasonable battery of model systems for the study of potential anticancer properties of a substance?

The battery should include both cellular and molecular assays, and the assays should address all of the known processes involved in cancer (unregulated cell growth, angiogenesis and metastases). Cellular assays have the advantage of being able to respond to an unknown mechanism of action since they contain all the replicative mechanisms found in most cells. The battery of cell lines should include a number of different cancer cell lines and also some normal cell lines. A variety of cell lines can assist in some of the problems associated with non-specific actions of anticancer drugs. Some examples of well established cell lines include melanomas, sarcomas, lung and colon squamous cell carcinomas, hormone dependent and independent breast cancer lines and hormone dependent prostate cancer lines. Human fibroblasts are used for selectivity evaluation. Activity in all of the cell lines may indicate a potential anticancer activity towards many types of cancer or it may indicate a non-specific cell cytotoxic response. Activity against one cell line may also mean selective activity towards one type of cancer. Drug resistance is also a major problem in anticancer treatments. Some drug resistant cell lines must also be included in the battery. All of these considerations impact on the final interpretation of the data from these assays.

A variety of enzyme level screens must also be used to complement and extend the ability of the test battery to confirm activity. These enzyme level screens are based upon the molecular knowledge of the mechanisms of cell growth, angiogenesis and metastasis. Topoisomerase I and II, protein kinase C, tubulin binding, DNA nicking, aromatase and C-17, 20 lyase assays, and steroid hormone binding assays are all examples of testing methodologies which should be included in a comprehensive evaluation of activity.

Batteries of tests are most useful for large random screening programs to identify quickly active ingredients and extracts upon which to base new therapeutic agents. However, are cellular and enzymatic assay batteries appropriate for ethnopharmacological evaluations? In most cases the ethnopharmacologist evaluates a limited number of ethnopreparations at any one time. It would not be cost effective or appropriate to establish the requisite assays to evaluate activity for one or a few ethnomedical preparations. In essence, will the investigator approach the ethnomedical evaluations as 'a series of assays in search of an active' or 'an active in search of an assay'?

The identification of the specific pathway or mechanism of action for an ethnomedical preparation is not required for the verification of efficacy. If the primary focus was to determine if the ethnomedicine should be supported in its use, the in vivo assays would be most appropriate and cost effective for initial testing. However, if the search is for a new drug from large numbers of preparations, then the battery approach may be better. However, the ethnopharmacologist must always question the possibility that an ethnomedicine may be acting by a new mechanism which would not be covered in the molecular assays.

The validation of ethnomedical activity is a difficult problem. Clinical evaluations would be the most desired method. However, such tests are not performed by local practitioners, who assume the activity and non-toxic nature of the preparations. They are also not performed by modern medicine which assumes inactivity or inability to provide an appropriate dosage form using the ethnomedical preparation (according to good manufacturing practices). The drug industry also has no interest in such studies because of the high cost of required preclinical studies which could not be recovered due to an inability to obtain patent rights and primary use in countries with minimal funds for health care.

Thus pharmacological models are the primary

methods used to validate ethnomedical activities. Animal models have been the foundation of such evaluations although in vitro systems are becoming the most recent focus. Both of these test systems can lead to active leads for consideration as therapeutic agents. However, the current trend to begin testing in in vitro methods begins the process of ethnomedical validation far from the pharmacologic model of man in which their reported activity was first found. Their identification as active agents in man will thus be even more unlikely due to the required need to again establish activity in animal models as the preparation returns to man through the gauntlet of animal models. There are obvious differences between man and animals in pharmacokinetic and pharmacodynamic processing of drugs which have not been discussed in the previous paragraphs and make success even more unlikely.

Ethnopharmacologists must work with scientific and governmental organizations to promote the study and use of ethnomedicines. The discovery of new agents for modern medicine and also for the use of ethnomedicines as an avenue for better health care for those who cannot afford the benefits of modern medicine is a difficult task dependent upon the selection of appropriate testing methodologies along the way to success. Ethnopharmacologists have the responsibility and opportunity to make important contributions to modern science through ethnomedicine.