Plants and Plant Products as Sources of Pharmaceuticals

20

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Human survival has always depended on plants. Early humans relied heavily on plants for food, medicine, and much of their clothing and shelter. The botanical skills of these early people should not be underestimated; all of the world's major crops were already domesticated in prehistoric times. The Age of Discovery was fostered by Europeans' explorations to find more economical trade routes to the East to bring back plant-derived spices and other products. Indeed, the first permanent contacts between Europe and the Americas 500 years ago was a direct consequence of that effort.

Aside from their value as sources of food, drugs, or industrial raw materials, plants are also important to humankind in many other ways. One can hardly imagine modern society without soaps and toiletries, perfumes, condiments and spices, and similar materials, all of plant origin, which enhance our standard of living. The roles of forests and other types of natural vegetation in controlling floods and erosion, in removing carbon dioxide from the atmosphere, and in providing recreational facilities are of immeasurable worth.

An adequate food supply is, and always has been, one of humanity's most pressing needs. Paralleling our need for food is our need for treatments for ailments. The practice of medicine today is very different from that of earlier times. This is largely because modern doctors have available a wide array of medicines with specific curative effects.

However, we still lack specific curative agents for a number of important diseases. Some 800 million to 1 billion people, nearly one-fifth of the world's population, suffer from tropical diseases: malaria, schistosomiasis, leprosy, leishmaniasis, etc. In the United States, heart disease, cancer, viral diseases (for example, AIDS), antibiotic-resistant infections, and many other ailments still lack adequate treatment.

Progress toward cures of the serious diseases that still afflict hu-

mankind depends upon discovery of new chemotherapeutic agents (drugs) which can effectively treat them. The search for new drugs has traditionally involved evaluating preparations of organisms (particularly higher plants) to look for appropriate biological activity. This is followed by the purification and characterization of the substance(s) responsible for the desired activity.

Effect of Discovering Quinine

This approach to drug discovery became commonplace after the 1820 discovery of quinine as the active ingredient in the antimalarial *Cinchona* bark. With the discovery and utilization of quinine as a pure chemical entity (drug), the course of modern drug discovery was irreversibly altered. An analysis of the historical development of the 20 most important pharmaceuticals utilized in the United States in 1988 reveals that in each case plants contributed an essential role, supplying either the actual medicines, leads for the medicinal chemists who developed the drugs, or precursors for preparation of the final medicines.

Efforts to develop new, clinically effective pharmaceutical agents have relied primarily on one of five approaches: (1) derivatization of existing agents, (2) synthesis of compounds similar to existing agents, (3) combination therapy of existing agents with other drugs, (4) improvements of delivery of existing agents to the target site, or (5) discovery of new prototype pharmaceutical agents.

While approaches 1-4 are important and need to be continued, there is an urgent need for the development of totally new prototype agents that do not possess the same toxicities, cross resistance, or mechanism of action as existing agents. Natural products have, in the past, provided a rich source of such compounds. It is essential that the search for new drugs continue to pursue this route. The major advantage of this approach is the likelihood of identifying new prototype drugs with quite different chemical structures and mechanisms of action and, hence, lower likelihood of similar toxicities and cross-resistance. Clearly, the higher plants represent a bountiful source of new prototypic bioactive agents that must be examined.

Bioassay Is Fundamental

The fundamental element of a drug discovery program is the bioassay(s) to detect the desired biological activity. The bioassay procedure selected for searching for new prototype drugs must meet a variety of criteria. In addition to ease of operation and low-to-moderate cost, the assay must show specificity and sensitivity to minute amounts of the agent being sought. Another important requirement of the assay is its ability to serve as a guide to selecting the agents showing activity for further purification. This is especially important in the screening of substances from natural sources, since these materials are likely to be in very low concentration in very complex mixtures. Only a combination of procedures meets these demanding criteria to serve as primary screens for biological activity.

Other important program elements must be coupled to the appropriate bioassay. The probability of selection and procurement of novel sources of potential preparations must be demonstrated. Also, it must be possible to adequately purify the active materials and to determine their structure. Initially detected activity must be confirmed in subsequent trials that help define the potential clinical utility of the substance. Finally, a "portfolio" of information about the substance must be accumulated in order to make a judgement about its potential for successful development into a clinically useful agent. For example, something must be known about such factors as its general toxicity, pharmacokinetics (how drugs are absorbed and eliminated from the body), mechanism of action, and analog development (which improves the clinical usefulness of the newly discovered chemical agent).

There are continuing efforts to discover and develop cancer chemotherapeutic agents from plants. Recent results show that certain chemicals found in select plants hold exciting promise for preventing or lowering the incidence of cancer. This is a specific example of how plant-derived chemicals will continue to contribute to the well being of humankind.

Discovery and Development of New Chemotherapeutic Agents

Plants have a long history of use in the treatment of cancer, though the majority of claims made for the efficacy of such treatment must be viewed with skepticism. Cancer is likely to be poorly defined in terms of folklore and traditional medicine, making it difficult to prove a specific treatment was effective. However, the National Cancer Institute (NCI) of the U.S. Public Health Service has recognized the value of plants as sources of potential anticancer agents. In 1960, NCI initiated a systematic effort to collect and screen plants for anticancer properties in collaboration with USDA. Between 1960 and 1982, some 35,000 plants were collected by USDA in over 60 countries and screened by NCI against a range of animal tumor systems.

A large number of chemical classes of plant products have shown activity in the animal tumor screens. Several plant-derived agents are now either in regular clinical use for the treatment of cancer victims or undergoing clinical evaluation. The best known of these agents are the so-called Vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus*. These drugs first became available in the 1960's, and are now used extensively, generally in combination with other agents, in the treatment of a wide variety of different cancer types.

With their use, long-term, disease-free survivals have been observed in the treatment of various lymphomas and leukemias, bladder cancer, and testicular cancer, and significant palliative benefits have been seen in patients with breast cancer, melanoma, and small-cell lung cancer. However, despite years of intensive research aimed at the viable synthesis of these agents, the cultivated plant is still the major source. Until recently, the phar-

maceutical company Eli Lilly was their major producer, using plants mass-cultivated for this purpose in Texas. With the expiration of Lilly's patents on these agents, cultivation and production ventures have been initiated in other countries.

Two other agents in clinical use are etoposide and teniposide, semisynthetic derivatives of podophyllotoxin, a lignan isolated from the Mayapple, *Podophyllum peltatum*, or from *Podophyllum emodii*. These agents show clinical activity against small-cell lung and testicular cancers, as well as lymphomas and leukemias. The starting materials for semisynthesis of these agents are isolated mainly from the Asian species *Podophyllum emodii*, which grows in the wild. However, supplies of this plant are reported to be dwindling, and it is possible that cultivation will be necessary to meet the demand for these agents.

The Promise of Taxol

The most recent addition to the cancer chemotherapy armamentarium is taxol. Taxol currently is isolated from the bark of the slow-growing Pacific yew, *Taxus brevifolia*. Significant clinical activity has been observed against refractory ovarian cancer, and substantial activity has been reported recently in the treatment of breast cancer. The supply of taxol needed to treat people with these two cancers in the United States alone is at least 100 kilograms per year. If preliminary activity observed against other serious cancers, such as lung cancer, is confirmed, the demand could exceed 300-400 kg. per year. It is clear that the bark of the Pacific yew will never meet these escalating demands, but taxol and related compounds, which can be converted into taxol, can be isolated from the leaves of other *Taxus* species.

Fortunately, various cultivars of *Taxus* species are popular ornamental shrubs in the United States, and a number of nurseries cultivate them on a large scale. NCI, in collaboration with USDA, Zelenka Nurseries, the University of Mississippi, and the Ohio State University, has initiated a program for the large-scale harvesting, drying, and extraction of the leaves of a common *Taxus* cultivar. Extracts will be processed to yield taxol and related compounds. It is anticipated that this renewable source will replace the *Taxus brevifolia* bark source in the next few years. This will relieve the pressure on this old-growth forest species and provide an important new source of income for growers of ornamental *Taxus*.

Taxus species have a distinguished history, and the historical and cultural significance of these magnificent trees has been recounted in a recent book, "The Yew Tree: A Thousand Whispers," by Hal Hartzell (1991). For centuries, Yew wood was used extensively in Europe in the manufacture of the longbows which played a dominant role in English victories in famous battles such as Agincourt. It seems ironic but appropriate that the role of these legendary plants has been transformed from that of use in the manufacture of implements of war to use in the manufacture of drugs in the war on cancer.

Another plant product of significance in the cancer chemotherapy program is camptothecin, an alkaloid isolated from the Chinese ornamental tree *Camptotheca acuminata*. Clinical trials of a soluble camptothecin salt were conducted in the United States in the 1970's, but were terminated due to observation of severe toxic effects. Recently, however, several new camptothecin derivatives have entered clinical development in the United States, Europe, and Japan. Preliminary results indicate activity against leukemias, lymphomas, ovarian cancer, and various lung cancers. The derivatives are prepared by semisynthesis from natural camptothecin supplied by Chinese and Indian sources. In collaboration with USDA, NCI is initiating a small cultivation project of *Camptotheca acuminata* to provide a domestic supply of the source raw material if needed.

Other plant-derived drugs undergoing clinical trials include homoharring-tonine, an alkaloid isolated from the small Chinese evergreen tree *Cephalotaxus harringtonia*, and phyllanthoside, a terpene glycoside isolated from the Central American tree *Phyllanthus acuminatus*. Homoharringtonine, obtained from sources in China, has shown activity against various leukemias. Phyllanthoside is in early

Alpana Joshi, a research associate at the Research Institute of Pharmaceutical Sciences, University of Mississippi, in Oxford, MS, manually injects a taxol sample extracted from ornamental yew clippings into a high-pressure liquid chromatography separator. The separator will analyze the sample for the amount of taxol present. *Bob Nichols/USDA 92BW0594-10*

clinical trials in the United Kingdom to determine the maximum tolerated dose in humans prior to advancing to trials against a range of cancer disease types.

Elliptinium, a semisynthetic derivative of the alkaloid ellipticine, is undergoing clinical trials in Europe. Activity has been reported against thyroid and renal cancer, and in the treatment of bone metastases resulting from advanced breast cancer. Ellipticine is isolated from species of the Apocynaceae family, including *Bleekeria vitensis, Aspidosperma subicanum*, and *Ochrasia* species.

NCI is continuing its collection program in tropical and subtropical regions worldwide, with the focus on rain-forests. The collections are being carried out through contracts with Missouri Botanical Garden (Central Africa and Madagascar), New York Botanical Garden (Central and South America), and the University of Illinois at Chicago (Southeast Asia). These collections were started in 1986, and are scheduled to continue until 1996. Over 4,000 dried plant samples per year are shipped to the NCI facilities in Frederick, MD, where extracts are prepared and tested for anticancer and anti-AIDS activity. A number of promising active compounds have been discovered, and these are being studied to determine whether they should eventually be advanced to clinical trials.

Development of Cancer Prevention Agents

At a recent university seminar on the topic of cancer chemoprevention by edible cruciferous plants, a member of the audience raised his hand and solemnly declared that he did not consider cruciferous plants like broccoli and brussels sprouts to be "edible." Even though consumption of these plants—broccoli, cauliflower, mustards, cabbage, radishes, and kohlrabi—is viewed with trepidation by some, in recent decades a strong link has been uncovered between them and decreased incidence of certain cancers. This protective effect has been confirmed in mice and rats, in which chemically induced tumors have been either reduced in number or prevented. This "chemoprotection" has been linked to an enhancement of detoxification systems in the body so that carcinogens are more rapidly metabolized and eliminated from the body.

Cruciferous plants contain substances called glucosinolates. Investigators have begun to focus their attention on the chemoprotective effects of specific glucosinolates and their breakdown products. Industrial rapeseed and crambe (see chapter 16.) are cruciferous oilseed crops grown to obtain high-erucic-acid oil. The meal remaining after extraction of the oil contains high levels (2-4 percent) of certain glucosinolates and their breakdown products, including nitriles and goitrins.

Historical Perspective

Three glucosinolate breakdown products are found at especially high levels in crambe and industrial rapeseed meals—cyanoepithiobutane (CHEB), cyanohydroxybutene (CHB), and vinyloxazolidinethione (goitrin). In

the 1970's and early 1980's, meals containing *high* concentrations of these compounds were found to have adverse effects on nonruminants when fed as a high proportion of the diet. However, the "negative press" resulting from these initial studies obscured the fact that these compounds are also present at *lower* concentrations in numerous edible crucifers, including cabbage and brussels sprouts. A curious effect was also observed—these compounds helped the body eliminate toxic materials, including those with glutathione (GSH) attached. GSH is a natural metalolic substance that attaches to reactive cancer-causing compounds for inactivation and elimination from the body.

Both CHB and goitrin at nontoxic doses induce enzymes that catalyze the deactivation of toxic compounds by GSH. CHB also induces GSH synthesis, particularly in the pancreas, but also in the liver and kidneys. This effect on the pancreas is of particular interest, since there is no effective treatment currently available for pancreatic cancer, a common, rapidly developing, untreatable, and uniformly fatal cancer. The way in which these compounds and their relatives enhance the GSH detoxification pathway suggests an important role in cancer chemoprotection for the cruciferous plants that contain them.

Status of Technology

The technology for efficient removal of glucosinolates from meal is already developed, but it is not cost effective at current prices for the oil and the meal. An important use and the resulting high value for the glucosinolate material would help offset the cost of removal. Research is under way on inexpensive techniques for extracting glucosinolates from the meal at the time of crushing using various water/solvent combinations.

Formation of the active breakdown products of glucosinolates is dependent on enzymes within the seed itself. Techniques have yet to be developed for large-scale extraction of these enzymes or for nonenzymatic breakdown of the extracted glucosinolates. Techniques for extraction and purification of the nitriles and goitrins exist, but must be refined for large-scale production if these compounds actually prove effective as chemopreventive or chemotherapeutic agents.

Successful technologies for cultivating, harvesting, and processing industrial rapeseed and crambe are being utilized in the few areas in the United States where these crops are now grown commercially.

Perhaps the biggest hindrance to production of pharmaceutical agents from industrial rapeseed and crambe is the long-term nature of the research that must take place before the glucosinolate products from these plants can be touted as chemopreventive or chemotherapeutic agents. Much needs to be learned about the metabolism, distribution, and mechanism of action of these compounds. Furthermore, studies are needed to ensure that these compounds themselves have no long-term adverse effects at the doses used for protection or treatment. A long-term commitment of time and funds to design and perform

the required studies is needed to optimize the chances for success.

The advantages for U.S. agriculture would be in those geographic areas where land is available, but where major crops cannot be or are not grown for economic, climatic, or other reasons. The economies of scale are such that local, small-scale processing plants for cheap extraction of oil and glucosinolates could be built and local markets for the meal could be utilized, thereby decreasing bulk transportation costs and revitalizing depressed local rural economies.

The potential for domestic production of a high-value "natural" class of important preventive and therapeutic agents is present, and preliminary data are promising, but much is still uncertain about the ultimate utility of these derivatives of industrial oilseeds. If one or more of these compounds were to prove efficacious in preventing or treating cancer (in particular colonic and pancreatic cancers), an important pharmaceutical use for glucosinolates derived from crambe and rapeseed would come into being. Both cancers are among the top 10 in this country, and pancreatic cancer is unremittingly fatal.

Summary

The examples above are drawn from a variety of efforts to find preventive or chemotherapeutic agents specifically for cancer and are only illustrative of the many research and development efforts under way to find new pharmaceutical prototypes from plants for the treatment of a host of other diseases as well. Research on artemisinin from *Artemesia annua* holds promise for development of therapy for drug-resistant malaria, a major emerging disease throughout the world. Preparations of *Ginkgo biloba* are being utilized to increase peripheral and cerebral blood circulation, and they hold promise for improvement of the health status of the elderly. In Europe, materials isolated from *Echinacea purpurea* are being developed as immune system stimulants. Successful development of such agents would represent a whole new approach to treatment and prevention of infectious disease.

These are just a few examples of the many efforts to find new pharmaceuticals from natural sources and to continue the history of the contributions of plants to human survival. ❑