Altern Med Rev 2001 Dec;6(6):567-79

Review of Antiviral and Immunomodulating Properties of Plants of the Peruvian Rainforest with a Particular Emphasis on Uña de Gato and Sangre de Grado

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Abstract

Viral diseases, including emerging and chronic viruses, are an increasing worldwide health concern. As a consequence, the discovery of new antiviral agents from plants has assumed more urgency than in the past. A number of native Amazonian medicines of plant origin are known to have antimicrobial and anti-inflammatory activity, although only a few have been studied for their antiviral properties and immunomodulating effects. Those most studied include: sangre de grado (drago) (Croton lechleri) in the Euphorbiaceae family and uña de gato (Uncaria tomentosa) in the Rubiaceae family. This article reviews the chemical composition, pharmacological properties, state of current research, clinical use, and potential antiviral and immunomodulating activity of these and other plants from the Peruvian Amazon. (Altern Med Rev 2001;6(6):567-579)

Introduction

Co-evolution between plants and their natural enemies including insects, bacteria, fungi, nematodes, animals, humans, and viruses is considerably more far reaching than current theories of reciprocal interactions suggest. Counter-resistance, genetic adaptability, polymorphic immune capacity, and pleomorphism among microbial agents allow for immense diversity of species and endless biochemical possibilities.[1] In order to adapt to environmental insults, plants produce a vast number of natural products that have antimicrobial and immunomodulating potential.[2] These include isoflavonoids, indoles, phytosterols, polysaccharides, sesquiterpenes, alkaloids, glucans, tannins, a variety of vitamins and trace minerals that function as antioxidants and co-enzymes, and many other phytochemical substances. In addition, there are a number of parallels between plant immunological activity and the immune systems of mammals, including adaptive mechanisms for viral resistance.[3]

Both the attribute of reciprocal natural co-evolution and the concept of shared chemistry among species are characteristics that allow humans to use plants as antiviral and immunomodulating

medicines.[4] In an age of emerging new viruses with stunning virulence, natural antiviral and immunomodulating substances could play a significant role in human disease prevention and treatment.

Amazonian Ethnobotany

The Amazonian region of northwestern Peru is among the earth's richest zones of biodiversity. It includes plants, animals, insects, as well as microbial organisms, and is one of nature's perfect evolutionary laboratories for plant biology. Ethnobotany has a long and distinguished history throughout the Amazon basin and its tributaries. Richard Evans Schultes (1915-2001), a tireless supporter of Amazonian ethnobotany,[5] began his investigation in the northwestern Amazon in 1941.[6] In previous centuries much of our knowledge of Amazonian flora is credited to Alexander von Humboldt of Germany (1769-1859) and Richard Spruce of England (1849-1863), as well as to Henry Hurd Rusby of Columbia University (1885-1928). Contemporary authorities on Amazonian ethnobotany include James Duke[7] and Mark Plotkin.[8]

Natural Product Selection

There are two ways natural products are selected for investigation. The classical method is laboratory based and relies on previous taxonomic findings, phytochemical factors, immunopharmacological studies, and random screening methods. The other, which is gaining popularity among investigators, is searching traditional texts and herbal medicine usage, including oral interviews with traditional indigenous healers the ethnobotanical route.[9,10] In one study, researchers found that an ethnobotanically driven approach led to a higher percentage of active compounds isolated than the standard high volume random screening method, reporting a 125-630 times more effective yield depending on the type of virus.[11] In another screening, 207 plants were tested for antiviral activity with 64 percent showing in vitro activity, 42 percent with strong activity.[12]

Antiviral Activity of Peruvian Plants

Largely due to the AIDS epidemic, an imperative for developing effective antivirals has generated considerable activity in anti- viral screening during the last two decades. However, the search for antiviral compounds has not been easy. Relatively few antiviral drugs are available, and those approved for use often have high side-effect profiles and exhibit the potential to cause rapid resistance among targeted viral strains. According to Colegate, an antiviral must meet three criteria: (1) it must inhibit the virus completely without affecting the host cells, (2) it must have a broad range of activity, and (3) it must not be immunosuppressive (Table 1).[13]

A number of plant substances have been found to meet the basic criteria, and screenings have been performed on several thousand plant extracts and other natural products. However, considering the overwhelming volume of medicinal plants in the upper Amazon and the near epidemic prevalence of hepatitis B,[14] as well as the high incidence of hepatitis A, D, C, E,[15] and G,[16] and other viruses like dengue strains in Peru, surprisingly few herbs from this region are listed in the literature as having traditional uses for viral diseases.

Selected antiviral plants of the Peruvian Amazon are listed in Table 2. Three have been well studied: Croton lechleri, Phyllanthus niruri, and Uncaria tomentosa. Among these, Croton and Phyllanthus have received more attention than Uncaria for their antiviral properties. Extensive research has been conducted on Curcuma, primarily for its anti-inflammatory effects[17] and on inhibition of HIV,[18] although primarily with species found in India and China. Mangifera has been studied for its anti-inflammatory[19] and antiviral activity against herpes simples II virus,[20] although primarily with Cuban, Chinese, or Indian species. Although considered to have antiviral activity and extensively used for ceremonial purposes among Amazonian healers, few studies have been performed on the antiviral properties of the tobacco plant, Nicotiana tabacum. Dr. Roberto Inchaustegei of Iquitos has reportedly used Draconitium loretense in combination with uña de gato for the treatment of AIDS, but this author was unable to find any published studies to confirm efficacy.[21] The Copaifera paupera tree produces an aromatic oleoresin that is made into oil that contains copalic acid and sesquiterpenes with reported anti-inflammatory properties,[22] and is applied topically to herpes blisters.

The Euphorbiacea family is found worldwide with 300 genera and 7,500 species identified to date. In one ethnobotanical study, 30 African Euphorbia species were studied for their antiviral activity.[23] This family also contains a number of medicinal herbs used in traditional Amazonian healing. In a 1988 study, 34 Amazonian plants belonging to this family were screened for antimicrobial activity, including against cytomegalovirus. Of those tested, 16 were found to have documented use as medicinal agents.[24] The two most well documented with antiviral activity are various Phyllanthus species[25] and Croton lechleri.

Several Phyllanthus species used for medicinal purposes occur in the Amazon, including P. acutiflolius, conami, diffuses, nobilis, and niruri.[26] In traditional Amazonian medicine, Phyllanthus species are primarily used to dissolve kidney and gall stones; therefore, the name chanca or quebra piedra which means literally "to break stone." Phyllanthus niruri, the most studied of Phyllanthus species, is an annual herb found in tropical countries around the globe and has been used in India for the treatment of jaundice for thousands of years. Because of its reported effectiveness in Ayurvedic medicine for hepatitis B, extensive research has been conducted on this herb. It is considered to have hepatoprotective[27] and anti-viral effects against hepatitis B,[28] HIV,[29] and possibly hepatitis C virus (HCV).[30]

Immunomodulating Activity

Immunomodulating activity refers to biological or pharmacological effects of compounds on humoral or cellular aspects of the immune response. The human immune response is a highly

complex and extraordinarily sophisticated system involving both innate and adaptive mechanisms.[31] Studies of how plant substances affect immune response employ mechanistic bioassay methodologies. Basic research on natural substances with immunomodulating properties is performed by assays primarily carried out on the stimulation of nonspecific immunity of the innate response, such as the efficiency of granulocytes, macrophages, complement, and natural killer cells, and their effects on phagocytosis, lymphocyte proliferation, and T-lymphocyte migration macrophage activation by beta-1,3-D-glucan[32,33] and natural killer (NK) cell augmentation by Echinacea purpurea for example.[34] More recent research on immunomodulating substances includes studies on cytokine production by macrophages such as interleukin-1 (IL-1),[35] interleukin-6 (IL-6),[36] and tumor necrosis factor-alpha (TNF-a).[37]

Plants from tropical rainforests represent a rich source of potential immunomodulating substances and leads from ethnobotanical practices have been the primary source of plant selection in recent years. Table 3 summarizes some of the most important immune modulating herbs of the Amazon basin. In one study, eight Amazonian herbs from the local region around Iquitos, Peru, with potential immune-stimulating effects were screened for phagocytic enhancement. Of those screened, three showed an increase in phagocytosis: Pistia startiotes, Piper peltatum, and Uncaria tomentosa.[38]

Phyllanthus niruri, as previously discussed, is best known for its antiviral properties, and at least two studies show related species may have potential immunomodulating activity.[25,39] Although there are more than 12 known Dracontium species, none have been studied for immunomodulating effects and little is known of the chemistry of the genus.[40] Pistia startiotes is an aquatic plant commonly seen floating on the Amazon River, its tributaries, and the multiple lakes of the region. A similar species is considered a nuisance weed in Cuba and Florida, where it can obstruct waterways due to its dense overgrowth. This plant has potent phagocyte-enhancing activity and has been used topically to treat warts and fungal conditions by local healers. Although Piper angustifolium and related Piper species have been studied for their antimicrobial effects,[41] the author was unable to find any references outside the Flores study noted above to any immunomodulating effects of Piper peltatum. The two most well studied of Peruvian herbs for their antiviral and immunomodulating actions are Croton lechleri and Uncaria tomentosa.

Sangre de Grado (Croton lechleri)

Croton lechleri is a large tree that grows in the upper Amazon region of Colombia, Ecuador, and Peru where the majority of the field research has taken place. A dark red resin, from which the name sangre de grado derives, flows easily from cuts in the bark and is used fresh or processed into a powdered dry extract of the resin for medicinal purposes. In traditional Amazonian medicine sangre de grado is used as an oral gargle for sore throat, as a vaginal antiseptic after childbirth,[42] topically as a hemostatic, and taken internally for wound healing. It is also used to improve gastrointestinal function, to protect the mucous membrane lining of the lower gastrointestinal tract, and to treat diarrhea. There is only one comprehensive review of the

research and literature[43] and two randomized controlled trials in human subjects have been performed on extracts of sangre de grado.[44,45]

Chemistry and Pharmacology

The known chemical composition of sangre de grado includes a considerable number of compounds including several simple phenols and diterpenes,[46] proanthocyanidins, phytosterols,[47] a dihydrobenzofuran lignan,[48] and the alkaloid taspine.[49] The pharmacological actions of sangre de grado include anti-oxidant potential, anti-inflammatory effects, anti-bacterial activity, antitumor potential, anti-diarrheal effects, wound healing, antifungal effects, and antiviral activity.[43,50,51] In at least one study, its phenolic and diterpene compounds demonstrated potent antibacterial activity against Bacillus subtilis and Escherichia coli.46 In a study designed to evaluate its gastrointestinal effects, Miller et al concluded that, "sangre de grado is a potent, cost-effective treatment for gastrointestinal ulcers and distress via antimicrobial, anti-inflammatory, and sensory afferent-dependent actions."[52] In the Chen study, antitumor properties were investigated in a Brazilian species (Croton cajucara)[53] as well as an Ecuadorian species.[46] The alkaloid taspine hydrochloride has been found to be the main cicatrizant or wound healing principle[54] and a potential anticancer agent.[43]

The anti-diarrheal action of sangre de grado has attracted the recent attention of researchers and several papers have been published on the mechanisms and effectiveness of this herb for diarrheal diseases. SP-303, a novel proanthocyanidin substance derived from the purified latex of Croton lechleri, has been shown to be effective in the treatment of persistent diarrhea associated with AIDS. One paper describes the results of a multi-center, phase II, randomized, double-blind, placebo-controlled study on 51 subjects using this substance over a period of four days. Patients in the SP-303 group experienced a statistically significant reduction in stool weight.[44] An in vivo mouse study (treating cholera-toxin related secretory diarrhea), examining the mechanisms of the action of SP-303, showed that it has an inhibitory effect on cAMP-mediated chloride and fluid secretion.[55]

Extracts of sangre de grado have been shown to have antiviral activity against influenza,[56] parainfluenza, herpes simplex viruses I and II,[46] and hepatitis A and B.43 In a multi-center, double-blind, placebo-controlled study, a topical preparation of SP-303 was used to treat recurrent genital herpes lesions in AIDS patients. Viral culture revealed 50 percent of the treated group and 19 percent of the placebo-treated patients became culture-negative at the end of the 21-day trial.[45] Although immunomodulating properties of sangre de grado have not been specifically elucidated, it is feasible that its anti-inflammatory, antimicrobial, and antioxidant activity[51] may provide non-specific immunomodulating effects as well.

Clinical Applications and Dosage

There is a wide range of potential applications for sangre de grado, including as a broad-spectrum anti-diarrheal agent from causes such as side effects of drugs, chemotherapy or radiation treatment, microbial infections of the intestine, traveler's diarrhea, and viral-induced diarrhea as in AIDS. It may also have other uses in gastrointestinal disorders such as irritable bowel syndrome and ulcerative diseases. Its cytotoxic effects make it a possible antitumor agent

and its cicatrizant properties provide wound-healing potential. In addition, the antimicrobial and anti-inflammatory effects of sangre de grado make it a useful compound in the clinical treatment of chronic viral diseases and as a natural antibacterial agent.

The recommended dosage of the standardized extract of SP-303, a patented proanthocyanidin oligomer, is 250-500 mg, two to four times daily or as needed. It is considered a moderately quick acting medication for diarrhea and benefits may be felt in a few hours with relief evident within 24 hours. Recommended dosages for tinctures range from 10-30 drops up to three times daily, and for dry extracts 20-60 mg mixed in water three times daily.

Uña de Gato (Uncaria tomentosa)

Uncaria tomentosa, or uña de gato (cat's claw), is the best known of the Peruvian medicinal plants and the most frequently represented in the literature. A woody vine containing a clear watery sap, it grows wild in the upper Amazon region of Peru and neighboring countries, and can reach several inches in diameter and 1,000 feet in height. The part used medicinally is the inner bark of the vine from which a boiled decoction is made or extracts produced. In recent years, extracts of the root have also been prepared commercially. Uña de gato is considered a sacred plant among the Ashaninkas and other indigenous Peruvian Amazonian tribes such as the Campo. According to the Austrian investigator Klaus Keplinger, the herb serves as a means of "regulating the physical and spiritual worlds" for these tribal groups.[57] From the perspective of ethnobotany, the higher a plant's status among native peoples, the more potent it often proves to be medicinally. Although its uses by native healers have been known for over fifty years, it was not until Keplinger began studying the properties of uña de gato in 1974 that it began to receive attention for its potential medicinal value. Keplinger's research eventually led to the development of several extracts with immunomodulating properties.[55-57]

There are no randomized controlled trials on human subjects utilizing uña de gato; however, to date, at least five review papers have been written on uña de gato. A 1999 systematic review paper by Keplinger et al described an analysis of 55 works summarizing ethnomedical and pharmacological uses.[57] In a 1998 British narrative review paper, Syrimis concludes, "Indeed, Uncaria tomentosa's broad therapeutic application suggests it is a worthy addition to the list of drugs used to treat the immune system."[58] In an online article by the Center for Alternative Medicine Research in Cancer,59 43 papers were reviewed of which 32 (74%) were applicable to cancer. Of these, 14 (44%) were retrievable and only three were human studies of which two were clinical outcome studies and the remaining one was a case study. This paper concluded that, although the results of the reviewed studies were very positive in terms of cancer regression, they did not provide substantial information on use in cancer and were not well documented. The remaining papers include brief narrative reviews by Murray,[60] Dharmananda,[61] Falkiewicz,[62] and others.

Chemistry and Pharmacology

The chemical composition of uña de gato includes 17 different alkaloids,[63] quinovic acid glycosides, tannins, flavonoids, sterol fractions,[64] and other compounds. James Duke's Phytochemical and Ethnobotanical Database lists 29 chemical constituents found in uña de

gato.[65] Since the publication of Duke's database several additional compounds have been isolated, including two new triterpenes.[66] The most investigated of the active constituents in uña de gato for immunomodulating and anti-inflammatory effects are pentacyclic oxindole alkaloids, which are reported to induce a yet unknown immune regulating factor.[67]

Indole alkaloids possess an indole ring in their structure, a versatile heterocyclic structure discovered in 1866, and found in a considerable number of medicinal products from plants.[68] Uña de gato contains a number of oxindole alkaloids[67,69] with one or more of six known isomeric pentacyclic oxindole alkaloids believed to be the main active component. Isopteropodine-HCl, isolated from the root, was shown to be the most potent of the tested compounds (Figure 1) while pteropodine, isomitraphylline, and isorhynchorphylline had weaker activity, and no immunomodulating activity was found in mitraphylline or rhynchophylline.[70] Other oxindoles found in uña de gato include uncarine F, uncarine F-N-oxide, speciophylline, and formaosine-N-oxide.[71]

The pharmacological actions of uña de gato include antioxidant properties, anti-inflammatory activity, immunomodulation, cytoprotection, antimutagenic properties, and antihypertensive effects, as well as possible prevention of cerebral ischemia.[72-74] Immunomodulating activity includes suppression of NF-kappa B,73 enhancement of B- and T-lymphocytes, stimulation of phagocytosis,[75] and enhancement of IL-1 and IL-6.[76] In a Peruvian study on rats, the investigators found that phagocytosis was increased when an extract was administered at a dose of 400 mg/kg.[77] In a study by Sandoval et al a water extract showed cat's claw to be, "a remarkably potent inhibitor of TNF-a. The primary mechanism for cat's claw's anti-inflammatory action appears to be immunomodulation via suppression of TNF-a synthesis."[73] A 1998 study showed that pentacyclic alkaloids weakly activated human B- and T-lymphocytes, and that tetracyclic oxindole alkaloids reduced the activity of pentacyclic oxindoles.[78] In another recent study, Swedish researchers using an aqueous extract treated radiation-induced DNA damage in rats. Results indicated "significant" repair of DNA breaks.[79] Based upon this research, it is generally accepted that the pentacyclic oxindole alkaloids are the principle immunomodulating agents in uña de gato.[80]

Clinical Applications and Dosage

Uña de gato has broad therapeutic potential, including the treatment of chronic viral infections, viral and bacterial co-infections in AIDS, cancer, the prevention of radiation damage, and in inflammatory disorders. Although there are no randomized controlled trials or published human outcome studies, some conditions reportedly improved by uña de gato include osteoarthritis, rheumatoid arthritis, prostatitis,[60,62] as a non-specific immuno-modulating agent in viral illnesses and cancer,[81] and it may also have potential as an immunomodulating adaptogen in aging.[79] Table 4 summarizes potential therapeutic uses of uña de gato and sangre de grado.

Several standardized extracts of uña de gato are available commercially including, (1) a root extract containing a minimum of 1.3-percent pentacyclic oxindole alkaloids free of tetracyclic oxindole alkaloids, with a recommended dose of one 20 mg capsule three times daily for the first ten days and then one capsule thereafter, and (2) a low-molecular-weight fraction hot-water extract from the whole plant containing eight-percent carboxyl alkyl esters with a suggested

dosage of 100 mg three times daily. The whole plant extract is considered a non-specific immunomodulating agent and may be used synergistically with antioxidant therapy.[82] The dosage for C-Med-100, a patented extract of Uncaria tomentosa bark standardized to eight-percent carboxyl-alkyl-esters, is 300 mg daily. A variety of other non-standardized uña de gato products are available, including powders from the whole dry inner bark of the vine, crude extracts, and various alcohol extracts from 1:1 to 8:1. As with sangre de grado, the most effective dosage for these substances remains unclear. To make a tea from the dry bark, Mejia recommends 100 grams per liter of water, slowly boiled for up to an hour or longer.21 For tinctures, the general dosage is 1 mL 1-3 times daily, and for dry extracts 500-2,000 mg mixed in water 1-3 times daily.

Toxicity and Drug Interactions of Uña de Gato and Sangre de Grado

Due to the bitterness of both of these herbs, mild nausea may occur upon ingestion of crude extracts or teas, but other than diarrhea reported in one study,[45] no other gastrointestinal events have been associated with these herbs. In recommended dosages, sangre de grado is considered non-toxic, and there are no known contraindications or drug interactions. Uña de gato is also considered non-toxic and more information is available on its safety than sangre de grado. In the Sheng study, the LD50 of a single dose of C-MED-100, a novel water extract of Uncaria tomentosa, was determined to be greater than 8 gm/kg.79 Santa Maria et al also determined uña de gato to be non-toxic by in vitro bioassays of Chinese hamster ovary cells and cells of the bacterium Photobacterium phosphorem.[83] In addition, historical ethnomedical usage and current use by health care consumers suggest low or no toxicity. However, due to potential immune stimulation, uña de gato should not be used in patients scheduled for organ transplants, skin grafts, during immunosuppressive therapy, and, although Murray states that uña de gato may be helpful in rheumatoid arthritis,[60] long-term use should be avoided in patients with autoimmune disorders until further information is available. Also, since the rhynchophylline alkaloids contained in uña de gato have antihypertensive effects, it may potentiate the action of antihypertensive drugs and their concurrent use should be avoided.[84] Until the effects of both of these herbs are better known, it is advisable to avoid their use in women attempting pregnancy, during pregnancy and lactation, and for children under three years old.

Discussion

Although the field of study in immune enhancing compounds is relatively new, natural products from plants represent a rich and promising source of novel molecules with immunomodulating properties. Prof. Hildebert Wagner of the University of Munich, one of the world's most distinguished researchers on adaptogens and immunomodulating agents from plants, including a significant amount of research on Echinacea[85] and Uncaria tomentosa,75 has published over 800 papers in this field, including a textbook on plant immune-modulating agents[86] Ultimately, two broad questions on the research of immunomodulating natural substances remain: (1) whether stimulation of innate response parameters translates into activation of the entire immune

system,[87] and (2) whether isolated pure compounds derived from plants have the same or similar immunomodulating properties as the whole plant preparation or their crude extracts.[70]

Plants are also a promising source of systemic broad-spectrum antivirals that may cause less damage to host cells infected by chronic viruses, such as hepatitis C, than do pharmaceuticals. Topical antiviral substances are also important areas of study for the treatment of viral lesions such as in herpes simplex virus, and plant-based substances offer promise as virucidals.

The most promising of the currently known Amazonian herbs are Uncaria tomentosa and Croton lechleri, with Uncaria the more studied. Both plants have similar therapeutic properties, including anti-inflammatory, antiviral, antibacterial, antioxidant, and immunomodulating activity. By current investigations, the therapeutic differentiation between the two is that uña de gato appears to have more immune-stimulatory effects with sangre de grado demonstrating more antimicrobial activity. However, with a few exceptions, the majority of studies have been in vitro or in animal models, with weak to moderate immunomodulating and antiviral effects. There is no overwhelming evidence to support the public perception of these herbs as potent immune stimulants, although they appear to be beneficial and safe, non-specific immunomodulating botanicals. Further research, both in the laboratory and clinically, is warranted.

Conclusion

The available research indicates that both sangre de grado and uña de gato have anti-inflammatory, immunomodulating, and antiviral effects that make them interesting candidates for further study,10 possible adaptogenic and cellular repair properties that have not yet been fully explored, and a broad range of potential therapeutic applications. With renewed concern over viral plagues, increasing incidence of cancer, and inflammatory conditions associated with aging, additional testing, both in vitro and in vivo, of these medicinal plants of the upper northwestern Amazon basin may prove valuable to the health of individuals and to nations.

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