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Review

Aloe-emodin novel anticancer Herbal Drug

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Abstract

The electrochemical behaviour of the anticancer herbal drug **emodin** hydroxyanthraquinone present in **Aloe vera** leaves has a specific **in vitro** and **in vivo** antineuroectodermal tumor activity. The compound does not inhibit the proliferation of normal fibroblasts n or that of hemopoietic progenitor cells. The cytotoxicity mechanism consists of the induction of apoptosis, whereas the selectivity against neuroectodermal tumor cells is founded on a specific energy-dependent pathway of drug incorporation. Natural compounds that have traditionally been used to treat a variety of diseases for hundreds of years (1, 2, 3) . We assayed only those natural compounds that have already been proven to be nontoxic, and we evaluated their efficacy against highly malignant tumors that are not normally included in the classical screening assays.

Keywords: Anticancer herbal drug; Emodin; Electrochemical; Dynamics parameters.

Introduction

Herbal medicines were one of the major resources for health-care in early eras. Currently, herbal medicines are gaining more attention from modern pharmaceutical institutes, as scientists become aware that herbal medicine is an almost infinite resource for drug development. Although the traditional Chinese medicine definition of cancer may differ from that of modern science, several Chinese plants do have significant antitumour activity. Emodin is one of these nature anticancer drugs, an active ingredient of the traditional Chinese medicine—rhubarb. At the time of diagnosis, 50% of affected children have disseminated neuroblastoma disease with a very poor prognosis that has remained unchanged in the last 3 decades (4, 5). Our study analyzed the cytotoxic potential of AE, a hydroxyanthraquinone (Fig. 1A) naturally present in the leaves of Aloe vera (6, 7). This report describes the selective in vitro and in vivo killing of neuroectodermal tumor cells by AE, the anticancer activity of which is based on apoptotic cell death,promoted by a tumor cell-specific drug uptake process that may off eropportunities for novel anticancer agents. The activities of in alternative cancer therapies, Induction of apoptosis is commonly reported among emodin and aloe-emodin, which involve disruption of mitochondria membrane potential, cytochrome c release, and activation of caspase 3. Emodin and aloe-emodin were also able to induce cell-cycle arrest, involving an increase in p53 expression level and accompanied by up regulation of p21.[1]

Chemistry: condensed and hydrolyzable tannins; chrysophanol, emodin, and physcion Medicinal Uses: Desert rhubarb is used topically for its astringency. External preparations are tightening to surface tissues and will lessen skin irritation and redness from burns, rashes, and scrapes. The plant, being moderately hemostatic is applied well to superficial cuts in order to staunch bleeding. Although symptomatic in effect, it can be almost miraculous in limiting the spread of stress or chemical sensitivity induced rashes, probably through its inflammatory-prostaglandin mediating properties.[2]

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Table: 1. Anticancer compounds derived from plant.

Plant source	Compound	Family
Allamanda cathartica	Allamandin	Apocynaceae
Ipomoea batatas	4-ipomeanol	Convolvulaceae
Penstemon deutus	Penstimide	Scrophulariaceae
Baccharis megapotamica	Baccharin	Compositae
Helenium autumnale	Helenalin	Compositae
Liatris chapmanii	Liatrin	Compositae
Phyllanthus acuminatus	Phyllanthoside	Euphorbiaceae
Vernonia hymenolepis	Vernolepin	Compositae
Gnidia lamprantha	Gnidin	Thymelaeaceae
Jatropha gossypiifolia	Jatrophone	Euphorbiaceae
Taxus brevifolia	Taxol	Taxaceae
Tripterygium wilfordii	Tripdiolide	Celastraceae
Brucea antidysenterica	Bruceantin	Simaroubaceae
Simarouba glauca	Glaucarubinone	Simaroubaceae
Holacantha emoryi	Holacanthone	Simaroubaceae
Marah oreganus	Cucurbitacin	Cucurbitaceae
Acernegundo	Acer saponin P	Aceraceae
Bersama abyssinica	Hellebrigenin	Melianthaceae
Acnistus arborescens	Withaferin A	Solanaceae
Combretum caffrum	Combretastin A-4	Combretaceae
Podophyllum peltatum	α-and β-peltatin	berberidaceae
P.hexandrum, P.peltatum	podophyllotoxin	berberidaceae
Steganotaenia araliaaceae	steganacin	umbelliferae
Jacaranda caucana	jacaranone	bignoniaceae
Stereospermum sauveolens	lapachol	bignoniaceae
Crotalaria spectabilis	monocrotaline	leguminosae
Heliotropium indicum	indicine-N-oxide	boraginaceae
Cephaelis acuminata	emetine	rubiaceae
Cyclea peltata	tetrandrine	menispermaceae
Thalictrum dasycarpum	thalicarpine	ranunculaceae
Fagara zanthoxyloides	nitidine	rutaceae
F.macrophylla	nitidine	rutaceae
Tylophora crebiflora	tylocrebine	asclepiadaceae
Acronychia baueri	acronycine	rutaceae
Ochrosia elliptica, O.moorei	ellipticine	apocynaceae
O.maculata	9-methoxyellipticine	apocynaceae
Camptotheca acuminata	camptothecin	nyssaceae
Cephalotaxus harringtonia	harringtonine	cephalotaxaceae
C.harringtonia	homoharringtonine	cephalotaxaceae
Catharanthus lanceus, C.	Leurosine	apocynaceae
C.roseus	vinblastine	apocynaceae
C.roseus	vincristine	apocynaceae
Maytenus buchananii	maytanacine	celastraceae
M.buchananii, M.serrata	maytansine	celastraceae
Maytenus buchananii	maytanvaline	celastraceae
Colchicum speciosum	colchicine	liliaceae
Bouvardia ternifolia	bouvardin	rubiaceae
B.ternifolia	deoxybouvardin	rubiaceae

Antineoplastic Effects: emodin suppresses tyrosine kinase activity of HER-2/neu-encoded pl85neu receptor tyrosine kinase resulting in antineoplastic effects. This is beneficial in controlling HER-2/neu overexpressing cancer cells (Zhang, 1998). Laxative (Cathartic) Effects: Anthraquinones such as Aloe are colonic-specific stimulant laxatives that have a direct action on intestinal mucosa, increasing the rate of colonic motility, enhancing colonic transit time, and inhibiting water and electrolyte secretion (Klinik et al, 1993; Godding, 1988)." - Thomson Healthcare, Inc., PDR for Herbal Medicines, Fourth Edition (Get the book.)

Materials and Methods

Drugs: AE was purchased from Sigma-Aldrich (Milan, Italy); it was dissolved in DMSO to reach a concentration of 200 mM and stored at 220°C. The AE is a fluorescent compound with a maximum excitation wavelength at 410 nm and a maximum emission wavelength at 510 nm. Alo in was a generous gift of MacFarlan Smith Ltd. (Edinburgh, Scotland). It was dissolved by slight warming in saline solution at the working concentration.

Table: 2.Cytotoxicity analysis of AE in Cell Culture:

Assay	Cytotoxic activity
animal	Female SCID mice
Organ	Neuroblastoma cells
Tissue	T-cell leukemia cells (CEM), and vinblastine-resistant cells (CEM VBL),
Disease	Neuroblastoma
Groth properties	Suspension

Cell Culture:

Neuroblastoma cells (IMR-32, IMR-5, AF8, and SJ-N-KP),pPNET cells (TC32), Ewing's sarcoma cells (TC106), T-cell leukemia cells(CEM), and vinblastine-resistant cells (CEM VBL), colon adenocarcinoma cells (LoVo 109), and doxorubicin-resistant cells (LoVo DX) were cultured in RPMI 1640 supplemented with 25 mM HEPES buffer and with 2 mM Lglutamine (all from Life Technologies, Ltd., Paisley, Scotland). The culture ofCEM VBL cells was supplemented with 10 mg/ml vinblastine (Lilly France, Saint-Cloud, Paris, France), and the culture of LoVo DX cells was supplemented with 0.1 mg/ml doxorubicin (Pharmacia, Milan, Italy). Cervix epithelioid carcinoma (HeLa) and human lung fibroblast (MRC5) cells were cultured in DMEM supplemented with 25 mM HEPES buffer and with 2 mM Lglutamine (all from Life Technologies, Ltd.). All culture medium was supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, Milan.Italy), 100 units/ml penicillin, and 100 mg/ml streptomycin (Sigma-Aldrich,Irvine, United Kingdom). All cell lines were grown at 37°C with 5% CO2humidified atmosphere.

In Vitro Cytotoxicity study:

The cytotoxic activity of AE was determined in exponentially growing cells in complete medium over 72 h. The cells were seeded in 12 wells/plate 24 h before the treatment; monolayer cells were plated at a density of 5–7 3 104 cells/well, and suspension cells were plated at 40 3 104 cells/well. AE was added to the experimental final concentration, and cells were counted 72 h later using the trypan blue exclusion assay. All of the experiments were conducted at least in triplicate.

Hemopoietic Progenitors and Neuroblastoma Colony Assay:

MNCsfrom BM aspirates and CB samples and from neuroblastoma cell lines (SJN-KP and AF8) were cultured in methylcellulose medium supplemented with a combination of recombinant colony-stimulating factors (Stem Cell Technologies, Vancouver, British Columbia, Canada). Cells were plated in triplicate at the concentration of 5 3 104/ml for BM- and CB-MNC, and 1 3 103 for NB cells, in 35-mm-diameter dishes (Becton Dickinson, Franklin Lakes, NJ) and incubated at 37°C in a 5% CO2 humidified atmosphere. MNC and NB cell lines were cultured in the absence or in the presence of different concentrations of AE. On day 14 of

culture, the number of CFU-GM and neuroblastoma Colonies was counted with an inverted microscope (Leitz-Diavert). All of the experiments were conducted at least three times.

Fluorescence-activated Cell Sorting Analysis: Neuroblastoma (SJ-N-KP), colon adenocarcinoma (LoVo 109), and cervix epithelioid carcinoma (HeLa) cell lines (1×10^6) were cultured for different time periods in the presence of AE or drug-free medium. Cells were harvested, washed twice with PBS, and fixed with cold 70% ethanol at 4°C. After centrifugation of the samples, propidium iodide (50 µg/ml in PBS) and RNase were added to the pellet for 20 min at 37°C to determinate the effect of AE on the cell cycle dynamics. DNA fluorescence was measured by flow cytometry (EPICS XL; Coulter, Miami, FL) analysis according to a published method [3] . To determinate drug uptake, SJ-N-KP, LoVo 109, and HeLa cells were cultured in the presence of 25µ M of AE or in drug-free medium at 37°C or at 4°C or in presence of NaN_3, for 24 h and then analyzed by flow cytometry [4] .

Two-Photon Excitation Microscopy: Neuroblastoma (IMR5), colon adenocarcinoma (LoVo 109), and cervix epithelioid carcinoma (HeLa) cell lines were seeded on microscope coverslips in 12-well plates and cultured with drug-free medium 24 h before treatment. Then AE was added at different concentrations. At different time points, cells were washed twice with PBS and examined by means of "fluorescence two-photon confocal microscopy." Optical sections were acquired with a TPE architecture described in detail elsewhere [5] .

Transmission Electron Microscopy Analysis: Cells were cultured with different concentrations of AE or with drug-free medium. At 24 and 48 h cells were scraped, washed twice in PBS, and fixed overnight at 4°C in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 6.9) and then processed according to Ciman **et al.** [6] . Ultrathin sections, cut with an ultramicrotome (Ultracut; Reichert-jung), were observed with the transmission electron microscope (TEM 300; Hitachi) operating at 75 kV.

Conclusion:

Aloe-Emodin was able to inhibit cell growth in several tumor cells, including human lung carcinoma, [10] hepatoma, [11] and leukemia cell lines. [13] aloe-emodin shows a high specificity for neuroectodermal tumor cells. [14] one of the important approaches for cancer chemotherapy is to regulate cell-cycle progression. G1/S cell-cycle arrest was found in human hepatoma, [12] glioma, [16] breast, [10] lung, [11] and colon [15] carcinoma cells upon treatment of rhubarb anthraquinones (emodin, [9] aloe-emodin, [13] and rhein [16]

The herbal medicines have great importance in the treatment of many diseases. Since herbal medicines are mainly used by Chinese, but now gaining acceptance all over the world and mostly in India. Herbal plants and their derivatives are widely used in the treatment of cancer. The treatment of cancer must include the benefits of botanical medicines. There are many classes of plant-derived cytotoxic natural products and the structural modification studies for further improvement and development of drug. New anticancer drugs derived from research on plant antitumor agents will be continuously discovered. The activities of flavonoids and the synergistic action shown by them with other drugs make them ideal in alternative cancer therapies. The chemopreventive effects that most flavonoids exert are likely to be the sum of their effect on several distinct mechanisms working inside the cell. The flavonoids have been focused for the research since 1930's but many of them have been used in traditional medicines for thousands of years in eastern countries. Anthraquinones are an important group of bioactive components found in many species of medicinal herbs such as rhubarb, senna, aloe and purslane. Induction of apoptosis is commonly reported among emodin and aloe-emodin, which involve disruption of mitochondria membrane potential, cytochrome c release, and activation of caspase 3. Emodin and

aloe-emodin were also able to induce cell-cycle arrest, involving an increase in p53 expression level and accompanied by upregulation of p21. This suggests that emodin could be a promising candidature for the research and development of new anti-tumor drugs.

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