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A New Class of Anti-HIV Agents from Medicinal Plants

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Abstract

Introduction

Human Immunodeficiency Virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS), is a member of the lentiviruses, a subfamily of retroviruses (1,2). Unlike other retro-oncoviruses, HIV is not known to cause cancer in humans and other animals, but it does present a formidable challenge to the host. The viral genome contains many regulatory elements that allow the virus to control its rate of replication in both resting and dividing cells. Most importantly, HIV infects and invades cells of the immune system and renders the patient susceptible to opportunistic infections and neo plasms.

It is estimated that two million people in the United States are currently infected with HIV, and between 5 to 10 million people are infected worldwide. Recent projections indicate that the majority of those now infected will develop AIDS within 7 year follow-up period. In 1993 alone, over 200,000 cases of AIDS have been reported domestically and more than half of these patients have died. It is apparent that AIDS is an unprecedented threat to national as well as global health. The search for effective therapies to treat AIDS is of paramount importance.

Plants as source of medicine

About three quarters of the world population rely mainly on plants and plant extracts for health care. Currently, more than 120 clinically useful prescription drugs are derived from plants. Most of these were developed because of their use in traditional medicine (3,4).

A new class of anti-HIV from medicinal plants

In the past several years, we have searched for antitumor and antiviral activities in a variety of plant extracts. From several hundred plants examined, we have identified, purified to homogeneity and characterized a new group of antiHIV agents from distinct and unrelated plant species. These include MAP 30 (Momordica Anti-HIV Protein, 30 kD) from the seeds of the bitter melon (5), TAP 29 (Trichosanthes AntiHIV Protein, 29 kD) from the root tuber of Trichosanthes kirilowii (6), GAP 31 (Gelonium Anti-HIV Protein 31 kD) from the seeds of Euphorbiaceae himalays, and DAPS 30 and 32 (Dianthus Anti-HIV Proteins 30 and 32 kD) from the leaves of the carnation (7). These compounds are unique in that they are capable of inhibiting not only the infection of new cells by HIV-1 but also the replication of the AIDS virus in already infected cells. In addition, these compounds have been shown

to affect the AIDS virus at levels which exhibit no apparent cytotoxic or cytostatic effects on normal human cells.

Anti-HIV activity

The anti-HIV activity of these proteins was measured in terms of their effects on viral infection and replication. Infection was measured by microtiter syncytium formation in the infectious cell center assay (8). Replication was measured by viral core protein p24 expression (9) and viral-associated RT activity (10).

The CEM-ss (syncytium sensitive, Leu-3 positive) cell line was used as the indicator cells for the microtiter syncytial-forming assay. The H9 cell line was used for p24 expression and viral-associated RT activity assays. HIV-1 virus was prepared and stocked according to established procedures (8). The cytotoxicity of these compounds was measured by their effects on cellular DNA and protein syntheses in uninfected cells.

All of the anti-HIV agents exhibited dose dependent inhibition of syncytium formation, p24 expression and HIV-RT activity. ID50s (inhibition dose at 50% inhibition) in the sub-nanomolar (0.2-0.3 nM) ranges were obtained for these agents. No cytotoxic or cytostatic effects were observed under the assay conditions. Cytotoxicity to uninfected cells in culture may be expressed as toxic doses 50 (TD50), the dose at which cellular protein and DNA synthesis is inhibited by 50%. This new class of plant-derived anti-HIV agents forms an important group of potential therapeutic drugs in the treatment of AIDS. Their therapeutic indices, defined as the TD50 divided by the ID50, are in the range of 1,000 to 10,000, using any of the three assays of antiviral activity. The potent anti-HIV activity and low cytotoxicity of these agents have been confirmed in human peripheral blood monocytes as well as in chronically infected macrophages.

Noval mechanism of antiviral action

We found that these anti-HIV agents possess dual capability to act against both DNA and RNA substrates. They exhibit a unique irreversible topoisomerase poison-like activity that converts supercoiled DNA into topologically inactive forms and interrupts DNA functions (11). These compounds also possess a N-glycosidase activity that acts specifically on the glycosidic linkage between the ribose and adenine or guanine at A4324 or G4323 of the 28S ribosomal rRNA, inactiviting the 60S ribosomal subunits and inhibiting polypeptide chain elongation (12,13). The ability of these anti-HIV agents to interrupt essential topological interconversions of DNA and ribosomal function of rRNA may provide novel mechanisms for their activity at multiple stages of the viral life cycle.

Amino acid sequencing, molecular cloning, crystallization and X-ray analysis

These anti-HIV agents are remarkable molecular devices that alter the fate of viral infection and tumorigenesis. They are striking in their specificity for viral-infected or tumor cells, and their lack of toxicity to normal human cells. We have determined the complete amino acid sequence of these proteins, cloned and expressed the genes encoding these agents. The recombinant products are as effective as their native counterparts in antiviral and anti-tumor actions. Recently, we have succeeded in the crystallization of these proteins.

Preliminary X-ray analyses of their crystals have been accomplished (14). The three-dimensional structure of these multifunctional molecules should provide valuable insight on their structure-function relationship.

Rational design of new drugs from traditional medicine

To define the action domains of these agents, we carried out structure-function mappings. We have mapped out an active oligopeptide of 33 amino acids. This oligopeptide is the minimal fragment necessary and sufficient for HIV-1 inhibition (15). It is also active in DNA binding, RNA binding and ribosome inactivation. This fragment is the smallest multifunctional peptide reported thus far. It is a unique tool for us to conduct molecular modeling and dynamic analysis. Conformational constraints can be incorporated to enhance activity and stability. Defined mimics with desired pharmacokinetic properties and bioavailability are being designed, synthesized and evaluated. Recently, through the Anti-Cancer Screening Program of the National Cancer Institute, we found that our anti-HIV compounds also possess potent anti-tumor activity against certain tumor cell lines (16).

In these studies, we are exploring new strategies for the prevention and treatment of AIDS and oncogenesis. If the basis of the anti-HIV and anti-tumor activities of these agents lies in selective interaction or uptake, this may be of use in engineering the delivery of these and other drugs for patient treatment. If the basis of their action lies in a fundamental biochemical difference between HIV-infected cells (or tumor cells) and normal cells, this difference may be investiand cancers. Rational design of novel drugs from traditional medicine offers new prospects in modern health care.

"It is an undoubted truth, that the less one has to do, the less time one finds to do it in. One yawns, one procrastinates, one can do it when one will, and therefore one seldom does it at all." -- Lord Chesterfield, Letters to his son