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Review

Plants as sources of antiviral agents

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Antivirals are substances other than a virus or virus containing vaccine or specific antibody which can produce either a protective or therapeutic effect to the clear detectable advantage of the virus infected host. The search for antiviral agents began in earnest in the 1950s but this was directed mainly by chance, with little or no scientific basis. It had a turning point in 1964 with a number of narrow spectrum agents whose values have been more difficult to establish. A lot of success has been achieved in the screening of plants for antibacterial, antifungal and antiviral actions. The use of plants or plant products, traditionally, as antiviral agents is relatively wider than their use in modern medicine. Some antiviral substances have so far been isolated from higher plants, algae and lichens. Suitable methods for evaluating antiviral properties of plants and their extracts include use of animal models, animal protection studies, egg inoculation studies and cell culture methods.

Key words: Antiviral, plants, lichen, algae, plant extracts.

INTRODUCTION

The term ‘antiviral agents’ has been defined in very broad terms as ‘substances other than a virus or virus containing vaccine or specific antibody which can produce either a protective or therapeutic effect to the clear detectable advantage of the virus infected host (Swallow, 1977). Unlike the search for antibiotics, which took root from the discovery of penicillin late 1930s, the search for antiviral agents began in the 1950s (Kinchington et al., 1995), but had a breakthrough in 1964 (Kucera et al., 1965). Early success in this direction included the use of methisazone for the prophylaxis of small pox and the use of idoxuridine for the treatment of herpes keratitis (Kinchington et al., 1995).

Two major obstacles to the development and use of effective antiviral chemotherapy are the close relationship that exists between the multiplying virus and the host cell, and that viral diseases can only be diagnosed and recognized after it is too late for effective treatment. In the first case, an effective antiviral agent must prevent completion of the viral growth cycle in the infected cell without being toxic to the surrounding normal cells (Desselberger, 1995). One encouraging development is the discovery that some virus specific enzymes are elaborated during multiplication of the virus particles and this may be a point of attack by a specific inhibitor. However, recognition of the disease state too late for effective treatment would render that antiviral agent useless even if they were available. Until early recognition of the disease state is provided, most antiviral chemotherapeutic agents will have their value as prophylactic agents.

The reason for the apparent lack of progress in antiviral chemotherapy as compared with the field of antibacterials has been a problem of selectivity (Kinchington, 1995). Any antiviral agent must selectively kill the pathogenic organism in the presence of other living cells. Sufficient biochemical differences exist between the metabolism of prokaryotic bacterial cells and mammalian cells to enable selectivity to be achieved, hence the early development of antibacterial agents, which were safe for human use. Viruses on the other hand, despite their apparent simplicity present a bigger problem. This is because during their replicative cycle, they become physically and functionally incorporated into the host cells and it is therefore difficult to distinguish unique biochemical features suitable for selective attack. Some viruses also persist in a latent infection (Cann, 1993 and Jawetz et al., 1989), in which case, antiviral drugs are less likely to be effective. However increased understanding of the

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molecular events of virus infections has meant that the search for antiviral drugs against specific targets can be conducted on a more rational basis.

TARGETS FOR ANTIVIRAL ACTIVITY

Viral envelope

The virus envelope is an outer lipoprotein bilayer membrane, which most viruses possess. Though some viruses contain lipid as part of a complex outer layer, these are not usually regarded as enveloped unless a bilayer unit membrane structure is clearly demonstrable (Cann, 1993). Enveloped viruses mostly bud through the host cell membrane leaving the cell intact and acquiring the envelope from the host cell membrane and with similar composition.

The viral envelope is advantageous to the virus possessing it. The structure underlying the envelope may be helical or icosahedral and may be formed before or as the virus leaves the cell. In the majority of cases, enveloped viruses use cellular membranes as sites allowing them to direct assembly, the formation of the particle inside the cell, maturation and release, in most cases being a continuous process. The envelope is effective as a protective layer preventing desiccation of, enzymic damage to the particle and through the surface glycoproteins foster recognition of receptor molecules on the host cell.

For enveloped viruses therefore, the viral envelope is a good target for antiviral chemotherapy because their destruction renders the virus vulnerable to destruction and the virus communicability less feasible. Detergents and ether had been known to solubilize or destroy lipid constituents of viral membranes; and ether susceptibility distinguishes enveloped and non-enveloped virus (Kinchington, 1995 and Jawetz et al., 1989). The following viral groups are enveloped and as such ether sensitive: herpes viruses, orthomyxoviruses, paramyxo-viruses, rhabdoviruses, coronaviruses, retroviruses, arenaviruses, togaviruses, flaviviruses and bunyaviruses.

Viral nucleic acid

On the basis of genome organization/nucleic acid component, two groups of viruses have emerged – the deoxyribonucleic acid (DNA) viruses and the ribonucleic acid (RNA) viruses. The nucleic acids are the seat of genetic information and direct all the processes relating to the viral propagation in host cells and their dissemination. In cell infected with DNA viruses, viral DNA serves as a template for its own replication and the synthesis of viral specific messenger RNA (mRNA). The enzymic synthesis of viral DNA may be catalyzed by either the DNA polymerase of the host cell or the virus. It seems likely that a viral RNA polymerase transcribes viral DNA into viral specific mRNA. RNA acts as template for its own replication as well as for the synthesis of viral specific proteins in RNA viruses. The RNA synthesis is required to form progeny RNA from parental RNA. The enzyme catalyzes the copying of the parental (plus) strand into its complementary (minus) strand, and the synthesis of new plus strand.

The implication of the above is that nucleic acids are and had been requisite targets for the design of antiviral chemotherapeutic agents, the most plausible approach being the formation of defective progeny nucleic acids which will be either unstable, give nonsense coding for viral proteins/enzymes, and do not maintain the virulence of the resulting virus.

Viral proteins

The discussion on the importance of viral nucleic acids as targets for possible viral chemotherapy overlaps with that of the viral proteins. This results from the very fact that the synthesis of viral proteins is not without the involvement of the nucleic acids and vice versa. However, the viral proteins present further unique opportunities for antiviral drug attack both as viral structural proteins as well as the functional proteins and enzymes. The mechanisms for the biosynthesis of viral coat proteins resemble the synthesis of normal cell proteins on the polyribosomes. Viral RNA acts as messenger for this synthetic process, which requires ribosomes, binding enzyme, peptide synthetase, translocase and possibly initiation and termination factors derived from the host cell. After completion of the synthesis, viral proteins are released form the ribosome.

The viral surface proteins play vital roles especially in the virus replication process where they aid virus attachment and penetration of host cells. The attachment phase of replication can be inhibited in two ways: by agents that mimic the viral attachment protein (VAP) and bind to the cellular receptor or by agents that mimic the receptor and bind to the VAP. Synthetic peptides are the most logical class of compounds to use for this purpose, but according to Cann (1993) there are considerable problems with clinical use of these substances, mostly high cost and poor pharmacokinetic properties of many of them. It is difficult to target penetration/uncoating stages of virus replication as relatively little is known about them.

Uncoating in particular is largely mediated by cellular enzymes and is therefore a poor target for intervention, although like penetration, one or more virus proteins often influence it.

Many viruses are known to have evolved their own specific enzymes to preferentially replicate virus nucleic acids at the expense of cellular molecules, and there is often sufficient specificity in virus polymerases to provide a target for an antiviral agent. This method has produced the majority of the specific antiviral drugs currently in use

(Kinchington, 1995; Cann, 1993). The majority of these drugs function as polymerase substrates (Roberts et al., 1990).

Other targets

In vitro studies have shown that short peptides and soluble $CD4^+$ can be used to block adsorption of HIV (Kinchington, 1995). Also, SP-303, an oligomeric proanthocyanidin form the latex of *Croton lechleri* has been show to exert antiviral effect on HSV and RSV by inhibiting viral adsorption and penetration through the plasma membrane (Ubillas et al., 1994). Amantidine has been found to block the later stages of virus assembly and release. The drug is thought to interfere with interactions between the viral membrane (M2) protein and the viral haemagglutinin therefore disrupting the envelopment of the nucleocapsid, hence blocking infectivity (Kinchington, 1995). Mutants resistant to amantidine through alterations in the M2 protein have been reported (Belshe et al., 1988).

There has also been increased attempt to synthesize antiviral agents that will stimulate the defense mechanism of the host. This is exemplified by the variety of biological response modifiers/interferon inducers that are at present under intensive study (Desselberger, 1995; Franz, 1989; Tomado et al., 1987). More recently, it has been shown that even mature virions can provide a target for antiviral drugs. The determination of the three dimensional structure of rhinoviruses has resulted in series of compounds (disoxanil – also known as the WIN series), which are targeted against mature extra cellular virions (Smith et al., 1986).

ANTIVIRALS FROM PLANTS

Apart from the importance placed by man on plants as source of food, their other great use has been in the area of medicine. The medicinal use of plants and their products dates back to antiquity (Ogunyemi, 1979). The World Health Organisation (WHO) has estimated that perhaps 80% of the inhabitants of the world rely chiefly on traditional medicine; hence plants and plant products have been in use in the treatment of infections many centuries before the active principles in the plant products could be elucidated through the improvements in science and technology.

Much success had been attained in the screening of plants for antibacterial and antifungal actions (Ogunlana et al., 1975 and Levan et al., 1979) than for antiviral actions due probably to the inability of the virus to be grown on artificial medium and the close relationship that exists between the multiplying virus and the host cell. But the failure of synthetic chemicals to effect cure of a wide range of viral diseases, and the frequency of viral resistance to the relatively few antiviral drugs currently used being on the increase and coupled with the successes achieved since antiquity in managing viral diseases with local medicinal herbs, have led to an increased interest in the search for antivirals from plants in recent years (McCutcheon *et al*, 1995), though success in this direction has been painstakingly slow.

Plant species that have been used in folk medicine or have been scientifically established to exert antimicrobial, antiviral and other biological activities are shown in Table 1. Even though a lot of these plants that have antiviral properties have not been used in modern medicine, their usage in traditional medicinal practice is fairly high. The significance of antiviral metabolites derived from plants and other higher forms of life, besides some direct medical success, is that they provide prototypes or templates for the organic chemist to use in the design of potentially superior new chemotherapeutic drugs.

Antivirals from higher plants

Higher plants in this context mean the common terrestrial vascular plants including ferns and liverworts, and the more properly byrophyta, pteridophyta and spermatophyta species (angiosperms, gymnosperms, monocots and dicots). Antiviral activity and/or cytotoxicity occur in a wider variety of plants and are not restricted to certain families as can be seen from Table 1. Plant tissue cultures have in recent times been found to have serious antiviral properties. More recently, cell cultures developed by biotechnological techniques have also been found to possess antiviral activities (Ibezim, 2003; Abonyi et al., 2000). The apparent lack of activity in certain families of plants may be due to small number of samples examined due partly to the difficulties associated with antiviral studies as compared with antibacterial investigations. So far, the largest number of non-microbial antibiotics, about 1300 substances has been isolated from higher plants (Berdy, 1982; Daziel, 1937; Ayensu, 1978; Irvine, 1961; Watt et al., 1962; Oliver-Bever, 1986; Iwu, 1986; Kokwaro, 1986).

Antivirals from algae

The term algae include all chlorophyll containing oxygen evolving photosynthetic organisms except bryophytes (mosses, liverworts) and vascular plants (Berdy, 1982). In size and complexity, they range from microscopic cells to giant kelps. These autotropic and thalophylic organisms occur in both fresh and seawater, but the majority of antibiotic compounds were isolated from seaweeds. At present, about 80 antimicrobially active algal products are known, the majority of which contain covalently bound bromine and are the products of red algae (Berdy, 1982). Most of these compounds are usually antibacterial agents active against Gram positive and rarely Gram negative bacteria but sometimes exhibit-

Table 1. Some antivirals from higher plants[1].

S/N	Antiviral compound	Chemical class	Plant source	Main action
1	Name unknown	Phytoalexine polysaccharide	*Solanium tuberosum, Phytophthora infestans*	Antiviral
2.	Phytolaecca American Protein (PAP)	Glycoproteid (Amphoter)	*Phytolacca Americana* *Phytolacca farmosus*	Polio, Influenza
3	Antiviral factor	Interferon 1	*Nicotiana glutinosum*	Antiviral
4	Lycoricidinol	N_2 containing heterocycline antibiotic (acidic)	*Lyconis radiata*	Antiviral
		,, (neutral)		
5	Lycoricidin	Alkaloid (basic)	*Lyconis radiata*	Antiviral
6	Chelerythrine	Alkaloid (basic)	*Chelidonium manis*	Antiphage
7	Emetine	Alkaloid (basic)	*Psychatria impacachuanha*	Antiviral (polis)
8	Okrobamine	Alkaloid (basic)	*Cabuncala spp, Striolata spp*	Influenza
9	Cryptopleurine	Alkaloid (basic)	*Bochmeria cylindrical*	Antiviral
10	5 – X	Pyran derivative (acidic)	*Clivia miniata*	Herpes, Polia
11	Elenoic acid	A-pyrone (neutral, acidic)	*Olives spp*	Antiviral
12	Parasorbic acid	Flavonol (acidic)	*Sorbus ancuporia*	Antiviral
13	Quercetin	Flavonol (acidic)	*Quercus spp, Prunus domestica*	Antiviral (polio)
14	Motin	Flavonol (acidic)	*Citrus spp, Chlorophora tinctoria*	Antiviral
15	Fisetin	Flavonone (acidic)	*Acacia spp, Rhus spp*	Antiviral
16	Naringenin	Flavonone	*Citrus spp, Acacia spp*	Antiviral
17	Hesperidin	Isoflavone	*Citrus spp, Citrus sinensis*	Antiviral
18	Rotenone	Condensed G-pyrone derivative (acidic)	*Balduina angustifolia* *Leucothac keikei*	Antiviral
19	Poriolide	Coumarin derivative	*Zizia aptera*	Antiviral
20	Angelicin			

[1]Adapted from Berdy (1982).

ing antifungal and anti-yeast activity, and in limited instances anti-tumour and antiviral effects.

Besides chondrid, a sesquiterpene isolated from *Chondria oppositiclada* and *Laurencia* spp., and a polysaccharide isolated from *Constantinea simplex* and *Forlowia mollis,* the chemical nature of the few known antiviral agents from algae have not been elucidated. Other algal antiviral agents have been isolated form 'kelp' algae, *Chlorella ellipsoidea, Dictyola* spp. and *Lyngbia majasculata* (Khatan, 1975; Robertson et al, 1977).

Antivirals from lichens

Numerous compounds isolated in the last few years from lichens proved to be active in certain physiological and pharmacological terms and a number of them were confirmed to be effective antimicrobial agents. These lichen products belong to the several distinct chemical classes including: polysaccharides, coumarone derivatives, lactone derivatives, orcinol and β-orcinol-type despsides and depsidones and long chain aliphatic oligocarboxy hydroxy acids (Esimone, 1997). Most of the lichen products are primarily active against Gram-positive bacterial and Mycobacteria, though extracts of *Parmelia capaerata, Evernia prunastri* and *Usnea spps* have been shown to be active against Gram-negative bacteria (Rowe et al., 1989). Some lichen polysaccharides and other lichen products such as psoronic acid, show antitumour activity, and usnic acid, the most widely distributed and best known lichen antibiotics has been used in several countries as a topical antibacterial agent for human skin diseases (Berdy, 1982), and is also reported to exert some antimitotic action, but at low concentrations, the acid displays a capacity to stimulate cell metabolism in some biological systems tested (Cardarelli et al., 1997).

Very little have been reported on the virucidal effect of lichen substances. Cohen et al. (1996) demonstrated that under optimal conditions of exposure to light, hypercicin (7,7-dichlorohypericin) and 5,7-dichloro-emodin from lichens exhibited strong inhibitory activity against herpes simplex virus type. Thus the antiviral activity appeared to be positively correlated with increasing substitution of chlorine in the anthraquinone structure.

METHODS OF EVALUATING ANTIVIRAL PROPERTIES OF PLANT EXTRACTS

Use of animal models

The earliest method for the study of viruses had been the use of animal host systems. Louis Pasteur for instance, began to study rabies in animals in 1881 and over a number of years he developed methods of producing attenuated virus preparations by progressively drying spinal cord of rabbits experimentally infected with the agent. Also through experimental transmission to mice, in 1900, Walter Reed demonstrated that yellow fever was caused by a virus and spread by mosquitoes (Cann, 1993). This discovery eventually enabled Max Thailler in 1937 to propagate the virus in chick embryo and to produce an attenuated vaccine – the 17 D strain that is still in use today. The success of this approach had led to increased use of animal systems to identify and propagate pathogenic viruses even with the adoption/ perfection of tissue culture technique.

Animal protection studies

As already indicated, the earliest method of virus study had been the use of animal hosts. In the area of antiviral chemotherapeutic research, animal models have been used either primarily as screening tools or applied in testing the efficacy of the test compound when it had been identified as effective/potent using any other method (Likar et al., 1977; Sloan et al., 1977).

Egg inoculation studies

Inoculation of fertilized hen eggs with the virus strain under study has been a powerful virological tool especially in the area of virus identification, vaccine production and in the evaluation of compounds for antiviral activities (Cann 1993; Jawetz et al., 1989). In the later event, after obtaining the virus titre and the cytotoxicity of the extract under study, different time-course studies are carried out using varying concentrations of the extracts. This depends on the study objective; that is, whether virucidal testing or inhibition of any stage of the virus replication is being investigated.

The use of egg inoculation in virological studies especially in developed countries is waning with the advent of faster and cheaper biotechnological tools.

Cell culture methods

Cell culture began in the 20th century with whole organ cultures, then progressed to methods involving individual cells, either primary cell cultures or immortalized cell lines which given appropriate conditions, continue to grow in culture indefinitely. In 1949, Enders and his colleagues were able to propagate poliovirus in primary human cell cultures. This opened what many regard as the 'golden age of virology', the identification and isolation during the 1950s and 1960s of many viruses and their association with human disease. Among the cell culture techniques are the plaque reduction techniques, viral cytopathic effect assay and haemagglutination inhibition techniques.

Conclusion

For long, plants used as whole plants, tissue cultures or cell cultures, have provided sources of antiviral agents. Other than plants, algae and lichens have also been employed as antiviral agents. Methods employed in screening plants and their extracts for antiviral activities include use of animal models, animal protection studies, egg inoculation studies and cell culture methods.

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