Effects of Animal Antiviral Chemicals on Plant Viruses

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ABSTRACT

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Tobacco mosaic virus (TMV) and cowpea chlorotic mottle virus were effectively inhibited by 14 of 27 chemicals reported to be active against several animal viruses. Adenine arabinoside, ribavirin, guanidine, cordycepin, tubercidin, (s)9-(2,3-dihydroxypropyl)adenine, distamycin A, 2,3-*bis*-(acetyl mercaptomethyl)-quinoxaline, cycloleucine, 3-deazauridine, 2,3-diaminopyridine, 8-azaguanine, 2-thiouracil, and 5-azacytidine were inhibitory. The ability of such a large proportion of the chemicals tested to inhibit both plant viruses argues for the possibility of a wide-spectrum antiviral compound for plant viruses. The selectivity of these compounds, measured as the concentration required to inhibit virus multiplication in leaf disks compared to the concentration that allowed growth and differentiation of tobacco tissue cultures, was low for most. Adenine arabinoside, ribavirin, (s)9-(2,3-dihydroxypropyl)adenine, and 5-azacytidine, however, allowed callus growth at concentrations greater than that required to inhibit virus multiplication in leaf disks, but these concentrations did not induce TMV-infected tobacco callus to grow free of TMV. Some of the tobacco callus cultures that grew on noninhibitory concentrations of cycloleucine or 3-deazauridine, however, became free of TMV.

Additional key words: chemotherapy, control, virus diseases.

Development and use of antiviral chemicals has enormous potential value to agriculture, particularly in perennial crops in which viruses cause chronic losses. If an antiviral chemical were to be produced commercially under present registration restrictions, it probably would have to control a number of viral diseases of economically important crops for the investment in registration to be recovered.

The purpose of this study is to examine the probability of finding antiviral chemicals that have broad-spectrum activity against plant viruses. This requires that a series of antiviral compounds be tested against a series of different viruses. One way to determine the spectrum of activity of chemicals against plant viruses is to test chemicals that have already been identified through extensive screening procedures to have activity against viruses of animals. A number of antiviral chemicals were tested against two virus-host combinations—tobacco mosaic virus (TMV) in tobacco and cowpea chlorotic mottle virus (CCMV) in cowpea. These virus-host combinations were chosen because they represent two different classes of RNA plant viruses, because they are easy to assay, and because further studies on modes of action of these chemicals will be more meaningful in the context of information already available on genetics and replication of these viruses. A substantial portion of the animal antiviral chemicals also inhibited both plant viruses. This argues for the possibility of a broad-spectrum chemical to control virus diseases of plants.

MATERIALS AND METHODS

Chemicals. Guanidine, cordycepin, tubercidin, cycloleucine, 3-deazauridine, 8-azaguanine, 2-thiouracil, hypoxanthine arabinoside, amantadine HCl, 5-azacytidine, 5-bromouracil, and 5-hydroxyuracil were obtained from Sigma Chemical Company, St. Louis, MO 63178. DHAP, uracil arabinoside, and benzimidazole-5,6-dichloro-1-β-D-ribofuranosyl were obtained from Calbiochem-Behring Corp., La Jolla, CA 92112. 2,3-Diaminopyridine, 2-(α-hydroxybenzyl)-benzimidazole, 5-nitrouracil, and cyclocytidine were obtained from Aldrich Chemical Co., Milwaukee, WI 53201. Distamycin A, rifampicin, and novobiocin were obtained from Boehringer, Mannheim, W. Germany. Ribavarin and phosphonoacetic acid were obtained from ICN, Irvine, CA 92715. Adenine arabinoside and pentostatin were gifts from Warner-Lambert Co., Detroit, MI 48232. BAMM Q was a gift from CIBA-GEIGY Ltd., Basle, Switzerland. Acycloguanosine was a gift from Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Culture and assay conditions. Strain U1 of tobacco mosaic virus was maintained in tobacco plants (*Nicotiana tabacum* L. 'Xanthi') and infectivity was assayed on 0.01 M potassium phosphate buffer, pH 7.0, and 1% Celite by the half-leaf method on *Nicotiana tabacum* L. 'Xanthi nc' with 6–12 replications of each inoculum in a random block design. Cowpea chlorotic mottle virus was maintained in cowpea [*Vigna unguiculata* (L.) Walp. 'California Blackeye'] plants, and infectivity of sap diluted with the above buffer was assayed by the half-leaf method on soybean (*Glycine max* L. 'Harosoy').

Test systems. At 4 hr after mechanical inoculation, disks 7 mm in diameter were removed from inoculated leaves. Ten disks per treatment were vacuum-infiltrated with the appropriate chemical solution and then allowed to dry on paper towels for 10–15 min. The disks were floated on the chemical solution in 3.5-cm petri dishes in a plant growth chamber at 25 C with a 14-hr photoperiod of approximately 15,000 lx. Control disks were similarly treated with distilled water. After 96 hr of incubation, all disks were removed from the chemicals and frozen at −20 C until infectivity was assayed as described above. Infectivity in chemical-treated disks was compared to that in water-treated disks. Each chemical was tested at two to four different concentrations differing fivefold, and each experiment was repeated at least twice. Data in Table 1 are reported as the lowest concentration tested that repeatedly inhibited virus infectivity accumulation by at least 90%.

Tissue culture procedures. Standard medium, to promote tobacco (*N. tabacum* L. 'Xanthi') callus growth, consisted of Murashige and Skoog (10) salts supplemented with 3 mg of indole acetic acid, 0.3 mg of kinetin, and 40 g of sucrose per liter of 0.8% agar. Regeneration medium was the same except that it contained 0.3 mg of indole acetic acid per liter and in addition 10 mg of N^6-isopentenyl-adenine per liter. Callus tissue was generated from pith tissues of tobacco plants infected with TMV. Chemicals were

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TABLE 1. Chemicals inhibitory to multiplication of tobacco mosaic virus (TMV) or cowpea chlorotic mottle virus (CCMV) in leaf disks

Chemical	Virus groups inhibited	Concentration (m*M*) required for 90% inhibition in leaf disks		Maximum concentration (m*M*) allowing growth of TMV-infected tobacco callus	TMV in[a] calli	TMV in[a] shoots
		TMV	CCMV			
Adenine arabinoside	Herpes virus, pox virus	0.6	0.6	1.0	+++	+++
Ribavirin	Broad-spectrum	0.2	0.1	0.4	+++	+++
Guanidine	Picornavirus	5.0	2.0	NT	NT	NT
Cordycepin	Broad-spectrum	0.2	0.2	NT	NT	NT
Tubercidin	Nonspecific	0.01	0.01	0.001	+++	+++
DHAP	Herpes virus, pox virus, rhabdovirus	...[b]	0.1	0.1	+++	+++
Distamycin A	Herpes virus, pox virus	...[c]	1.0	NT	NT	NT
BAMM Q	Broad-spectrum	0.015	0.03	0.03	+++	+++
Cycloleucine	Rhabdovirus	1.0	1.0	0.5	+++[d]	+
3-Deazauridine	Nonspecific	1.0	0.1	0.1	++	++[e]
2,3-Diaminopyridine	Picornavirus	1.0	1.0	0.1	+++	+++
8-Azaguanine	Nonspecific	2.0	2.0	<0.1	NT	NT
2-Thiouracil	Picornavirus	1.0	Not inhibitory	0.1	+++	+++
5-Azacytidine	Nonspecific	...[f]	0.1	1.0	+++	+++

[a] +++ => 100 Local lesions per half leaf (range of infectivity in control tissue); ++ = 10–100 lesions per half leaf; + = 1–10 lesions per half leaf; NT = net tested.
[b] 1 m*M* DHAP inhibited TMV 60%.
[c] 1 m*M* distamycin inhibited TMV 50%.
[d] Five of 26 calli had no detectable virus.
[e] Nine of 10 shoots tested had no detectable virus or mosaic symptoms as plants developed.
[f] 0.1 m*M* 5-azacytidine inhibited TMV 70%.

TABLE 2. Chemicals not inhibitory against multiplication of tobacco mosaic (TMV) or cowpea chlorotic mottle (CCMV) viruses

Chemical	Maximum concentration (m*M*) tested	Virus groups inhibited
Acycloguanosine	1.0	Herpes virus
Uracil arabinoside	1.0	Herpes virus
Hypoxanthine arabinoside	1.0	Herpes virus
2-(α-Hydroxybenzyl)-benzimidazole	0.5	Picornavirus
Phosphonoacetic acid	0.1	Herpes virus
Amantadine HCl	1.0	Influenza virus
Rifampicin	0.1	Pox virus, retrovirus
5-Bromouracil	1.0	Nonspecific
5-Nitrouracil	1.0	Nonspecific
5-Hydroxyuracil	1.0	Nonspecific
Benzimidazole-5,6-dichloro-1-β-D-ribofuranosyl	1.0	Influenza virus
Novobiocin	0.1	Herpes virus, pox virus, picornavirus
Cyclocytidine	1.0	Retrovirus

filter-sterilized and added to each medium after autoclaving. Peripheral areas of calli that grew in the presence of chemicals were removed in a manner to avoid taking any of the original seed callus and were assayed for infectivity of TMV as described above. New shoots that developed on media containing the chemicals were removed and assayed similarly.

RESULTS

Twenty-seven compounds reported to be inhibitory to animal viruses were tested for ability to inhibit the multiplication of TMV and CCMV. Results are in Tables 1 and 2. Compounds were listed as inhibitory only if they inhibited virus multiplication at least 90%. Of the 27 chemicals tested, 14 strongly inhibited at least one of the plant viruses.

The degree of selectivity of inhibitory compounds was estimated by comparing the concentration required to inhibit the viruses in leaf disks to the concentration that was toxic to the host. Toxicity was determined by recording the maximum concentration of a chemical that allowed growth of TMV-infected tobacco callus when the chemical was incorporated into solid medium. Moreover, callus cultures that grew in the presence of the chemical were transferred to a shoot-inducing medium containing the same concentration of the chemical to determine whether regeneration into organized tissues could occur. The presence of TMV in newly developed callus or shoots that grew in the presence of the chemicals was assayed to determine whether the chemicals eliminated TMV from the tissue cultures.

Adenine arabinoside. Adenine arabinoside blocks herpes virus replication with a high degree of specificity (9). It has not been reported to effectively inhibit RNA viruses in animal systems. This chemical effectively inhibited the multiplication of both TMV and CCMV. The selectivity of this compound was better than most of the compounds tested in that tobacco callus grew and differentiated on concentrations (1 m*M*) higher than that required to inhibit the virus in leaf disks (0.6 m*M*). However, the compound appeared to inhibit TMV less in callus tissues than in leaf disks. The amount of TMV in callus tissues that grew on this compound (1 m*M*) was not appreciably reduced (Table 1).

In animal cells, adenine arabinoside is rapidly deaminated by adenosine deaminase to hypoxanthine arabinoside that has substantially less antiviral activity (9). Thus, a deaminase inhibitor, pentostatin, is used in combination with adenine arabinoside to preserve activity. Deamination of adenine arabinoside appeared not to occur in the plant tissues. Pentostatin (0.25–1.0 μg/ml) had no effect on the activity of different concentrations of adenine arabinoside on inhibition of either TMV or CCMV in leaf disks or tobacco callus (*unpublished*). Also, the deaminase product, hypoxanthine arabinoside, was inhibitory to the multiplication of neither TMV or CCMV (*data not presented*).

Ribavirin. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide:virazole), a guanosine analogue in which the purine ring is open, inhibits the replication of a number of DNA and RNA viruses of animals. This chemical, which previously was shown to inhibit a number of plant viruses (6,7,11,12), also inhibited TMV and CCMV multiplication in these systems (Table 1). The multiplication of CCMV was more sensitive to ribavirin than was TMV. Tobacco callus grew and differentiated on a concentration of ribavirin twice that required to inhibit TMV multiplication in leaf disks. Newly developed tobacco callus or shoots still contained TMV, however.

Guanidine. We previously showed that this compound inhibits the multiplication of both TMV and CCMV (2,4). In preliminary experiments, guanidine was extremely toxic to growth of callus cultures and was not further tested.

Cordycepin. Cordycepin (3′-deoxyadenosine) inhibits RNA polymerization nonspecifically at higher concentrations by causing

chain termination due to the lack of the 3′ hydroxyl group. This chemical inhibited the multiplication of both TMV and CCMV. Because of its severe toxicity, it was not tested further.

Tubercidin. Tubercidin (7-deazaadenosine) nonspecifically inhibits RNA synthesis in viral, prokaryotic, and eukaryotic systems. It inhibited both TMV and CCMV at a relatively low concentration (10 μM), but only allowed callus growth at a concentration 10-fold lower, which did not inhibit TMV accumulation in callus (Table 1).

DHAP. DHAP [(s)-9-(2,4-dihydroxypropyl)adenine], an adenosine analogue in which the sugar moiety is replaced by an aliphatic chain, inhibits a number of DNA viruses and rhabdoviruses but fails to inhibit a number of plus-stranded RNA viruses in animal systems (5). This compound effectively inhibited the multiplication of CCMV, but that of TMV was inhibited substantially less. DHAP allowed growth and differentiation of tobacco callus at the same concentration that inhibited CCMV in cowpea leaf disks.

Distamycin A. This chemical inhibits the multiplication of several large DNA viruses of animals with some selectivity by binding to double-stranded DNA. Distamycin A effectively inhibited multiplication of CCMV but not that of TMV. It was toxic to callus tissues and was not tested further.

BAMM Q. BAMM Q [2,3-*bis*-(acetylmercaptomethyl)-quinoxaline], which is not a nucleoside analogue, inhibits poliovirus (1) and also inhibited both TMV and CCMV in leaf disks at relative low concentrations that were the same as or lower than concentrations that allowed callus and shoot growth. However, the chemical did not reduce TMV accumulation in tobacco tissue cultures that grew on media containing this compound.

Cycloleucine. This compound is another non-nucleotide analogue that inhibited the multiplication of both TMV and CCMV. Cycloleucine, which is an analogue of the amino acid methionine, inhibits the methylation of vesicular stomatitis virus RNA (8). The concentration that inhibited virus multiplication in leaf disks prevented tobacco callus growth. However, a lower concentration that allowed callus growth resulted in a few callus clones that were free of TMV. In contrast, in the other calli, the virus concentration was not reduced measurably. Shoots that developed contained reduced amounts of virus, but none of the shoots tested was free of TMV.

3-Deazauridine. This is a nucleoside analogue with little specificity in animal systems. It inhibited CCMV at 0.1 m*M* and TMV at 1.0 m*M*. Although tobacco callus grew on only 0.1 m*M* 3-deazauridine, the compound reduced the amount of TMV in both callus tissue and shoots, and most (9 of 10) of the resulting shoots were free of virus.

2,3-Diaminopyridine. 2,3-Diaminopyridine inhibited both TMV and CCMV but was relatively toxic to callus growth and did not reduce TMV in tissue cultures.

8-Azaguanine. This chemical inhibited both viruses but was severely toxic to callus growth.

2-Thiouracil. 2-Thiouracil effectively inhibited TMV without inhibiting CCMV as had been reported previously (3). Concentrations of this compound that allowed tobacco callus growth did not appreciably reduce the amount of TMV in treated callus.

5-Azacytidine. This compound inhibited the multiplication of CCMV more than that of TMV. It did not reduce the amount of TMV in tobacco callus tissue growing on the compound.

Table 2 lists a number of chemicals that are inhibitory to animal viruses that did not inhibit multiplication of TMV or CCMV by 90%.

DISCUSSION

A high percentage of chemicals reported to be inhibitory to viruses in animal systems were also found to inhibit the multiplication of two RNA viruses of plants representing two different virus groups. It was not surprising that several of the nonspecific inhibitors (corcycepin, tubercidin, 3-deazauridine, 8-azaguanine, and 2-thiouracil) also inhibited plant viruses. It also was not surprising that several of the picornavirus-specific inhibitors (guanidine, BAMM Q, and 2,3-diaminopyridine) inhibited TMV and CCMV. It was surprising that compounds that are very selective for DNA viruses in animal systems inhibited these RNA plant viruses. Adenine arabinoside, which has not been reported to inhibit an RNA virus of animals, effectively inhibited the multiplication of both TMV and CCMV. DHAP, which does not inhibit many plus-stranded RNA animal viruses, also inhibited both plant viruses. The ability of such a large proportion of the chemicals tested to inhibit both plant viruses, however, argues for the possibility of a broad-spectrum plant antiviral chemical. Only 2-thiouracil, DHAP, distamycin-A, and 5-azacytidine inhibited one virus without effectively inhibiting the other.

None of the chemicals tested in this study has enough selectivity to be useful in controlling virus diseases. For most of the chemicals that were inhibitory, the concentration required to inhibit the virus in leaf disks was higher than the concentration that allowed tobacco callus cells to grow and differentiate. Adenine arabinoside, ribavirin, BAMM Q, and 5-azacytidine were the most selective chemicals. Yet, even with these chemicals, the concentrations in which cells grew in culture were not appreciably lower than that required to inhibit virus multiplication in leaf disks. More importantly, these chemicals did not inhibit TMV accumulation in tobacco tissue cultures at the same concentrations in which they were inhibitory to TMV in tobacco leaf disks. An anomaly was that growth of TMV-infected callus cultures on cycloleucine or 3-deazauridine, two chemicals with relatively low levels of selectivity, resulted in calli or shoots that were free of TMV. A factor, perhaps as important as selectivity, is a combination of developmental conditions that allow the chemical to be more effective. Shepard (12) has shown that ribavirin treatment resulted in eradication of potato virus X when added during initiation of shoot morphogenesis, but not when added to protoplasts or proliferating calli. This area needs more study.

This study identified a number of new inhibitors of multiplication of plant viruses. Continuing studies on the mode of action of these compounds in inhibiting plant viruses hopefully will identify targets available for suppression of viruses in plants and will allow a logical approach to identifying chemicals that will be the next generation of plant antivirals.

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