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Antiviral Activities of 5-Ethynyl-1-β-D-Ribofuranosylimidazole-4-Carboxamide and Related Compounds†

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A series of novel compounds, 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides, have been identified as broad-spectrum antiviral agents. 5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR), the most potent congener of the group, showed antiviral potency about 10- to 100-fold greater than that of ribavirin. Similar in spectrum to ribavirin, EICAR was particularly active (50% inhibitory concentration, 0.2 to 4 μg/ml) against poxviruses (vaccinia virus), togaviruses (Sindbis and Semliki forest viruses), arenaviruses (Junin and Tacaribe viruses), reoviruses (reovirus type 1), orthomyxoviruses (influenza A and B viruses), and paramyxoviruses (parainfluenza virus type 3, measles virus, subacute sclerosing panencephalitis virus, and respiratory syncytial virus). EICAR was also cytostatic for rapidly growing cells (50% inhibitory concentration, 0.2 to 0.9 μg/ml). EICAR inhibited vaccinia virus tail lesion formation at doses that were not toxic to the host. EICAR is a candidate antiviral drug for the treatment of pox-, toga-, arena-, reo-, orthomyxo-, and paramyxovirus infections.

The only antiviral drugs which have been licensed for the treatment of respiratory virus infections are amantadine (rimantadine) in the prophylaxis or early therapy of influenza A virus infection and ribavirin in the therapy, by aerosol, of severe respiratory syncytial virus (RSV) infections in children (8). Ribavirin was described almost two decades ago as a broad-spectrum antiviral agent (33). It has shown clinical efficacy against influenza A and B virus, RSV, and parainfluenza virus infections (15) and Lassa fever (26). In the treatment of orthomyxo- and paramyxovirus infections, and in particular, RSV infections in children, ribavirin can be administered as a small-particle aerosol (23).

Among the ribavirin derivatives that have been described, the 3-carboxamidine (21, 39), 3-thiocarboxamide (39), 3-carboximidate (21), 2′(3′)-*O*-methyl (14), 4′-thio (29), and 3′-amino (27) derivatives, only the 3-carboxamidine derivative exhibits an antiviral activity spectrum that is similar to that of ribavirin. Thiazole C nucleosides (35) and diazaphosphole nucleosides (30), which are related to ribavirin, have also been synthesized. The thiazole C nucleoside tiazofurin (35) and its selenium analog selenazofurin (36) exhibit not only broad-spectrum antiviral activity (22) but also potent antitumor activity (3, 19).

Among the imidazole-4-carboxamide ribonucleosides (11, 37, 40), 5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide (FICAR) has shown a similar activity spectrum but lower potency than those of ribavirin (11). FICAR can be considered a close analog of ribavirin, whereby the N at position 2 of the triazole ring is replaced by a C-F group. We have designed a new series of imidazole derivatives, namely, 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides (25, 26a), which, again, can be viewed as close analogs of ribavirin, whereby the N at position 2 of the triazole ring is replaced by an alkynyl-carbon moiety (Fig. 1). 5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) is the prototype of this class of compounds.

MATERIALS AND METHODS

Compounds. The synthesis of the 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides (compounds 1, 2, 3, 4, and 5 [Fig. 1]) has been described previously (25). Compound 1 corresponds to EICAR (molecular weight, 267). Carbocyclic 3-deazaadenosine (C-c^{3}Ado) was provided by J. A. Montgomery (Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.). Ribavirin (Virazole) (molecular weight, 244) was obtained from ICN Pharmaceuticals (Costa Mesa, Calif.). Tubercidin was obtained from Sigma Chemical Co. (St. Louis, Mo.). The nucleosides (guanosine, adenosine, inosine, cytidine, and uridine) were also obtained from Sigma.

Radiochemicals. The radiolabeled precursors [*methyl*-^{3}H]-2′-deoxythymidine ([*methyl*-^{3}H]dThd), [5-^{3}H]uridine ([5-^{3}H]Urd), and [4,5-^{3}H]leucine ([4,5-^{3}H]Leu) were used to monitor cellular DNA, RNA, and protein synthesis, respectively, and were obtained from Amersham (Bucks, United Kingdom). Their specific radioactivities were 40, 30, and 52 Ci/mmol, respectively.

Viruses. The origins of the viruses and the preparation of the virus stocks have been documented in previous reports, as follows: herpes simplex virus type 1 (strain KOS) (10), herpes simplex virus type 2 (strain G) (10), cytomegalovirus (CMV) (strains Davis and AD-169) (34), vaccinia virus (11), vesicular stomatitis virus (VSV) (6), coxsackievirus type B4 (11), poliovirus type 1 (11), parainfluenza virus type 3 (6), reovirus type 1 (6), Sindbis virus (11), Semliki forest virus (6), Junin virus (1), Tacaribe virus (1), influenza A virus (strain Ishikawa) (32), influenza B virus (strain Singapore) (32), measles virus (strain Sugiyama) (17), subacute sclerosing panencephalitis virus (strains Niigata-1 and Kitaken-1)

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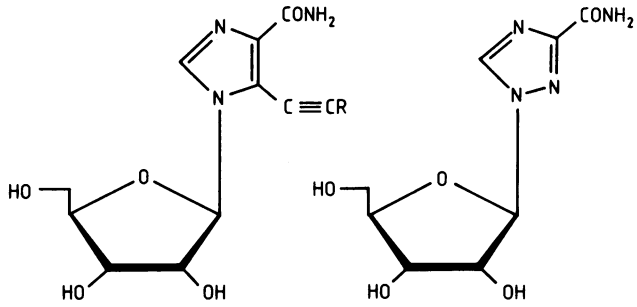
† This paper is dedicated to the memory of Prof. T. Ueda.



R =		
H	1 (EICAR)	Ribavirin
CH_2OH	2	
CH_2CH_2OH	3	
$CH_2CH_2CH_3$	4	
Phenyl	5	

FIG. 1. Formulas of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides and ribavirin.

(17), RSV (strain Long) (20), and human immune deficiency virus type 1 (strain HTLV-III_B) (28). The vaccinia virus stock used for the animal experiments originated from calf lymph; it was provided by the Rijksentstofinrichting (Brussels, Belgium). Its titer was 1.5×10^8 PFU/ml, and it was stored at 4°C.

Cells. The cell lines used for the antiviral activity assays were primary rabbit kidney (PRK), human embryonic lung (HEL) fibroblast, HeLa (a human epithelial cell line derived from a cervical carcinoma), Vero (a simian fibroblast cell line derived from African green monkey kidney), Madin-Darby canine kidney (MDCK), E_6SM (human embryonic skin muscle) fibroblast, BSC-1A (a simian epithelial cell line derived from African green monkey kidney), RK-13 (a rabbit kidney cell line), Georgia bovine kidney (GBK), CV-1 (a simian fibroblast cell line derived from African green monkey kidney), L-929 (a murine fibroblast cell line derived from an adult C3H mouse and mutagenized with methylcholanthrene), and MT-4 (a human T-cell line established by cocultivating normal human cord blood leukocytes with leukocytes from a patient with adult T-cell leukemia). The cells were grown in Eagle minimum essential medium supplemented with 10% fetal bovine serum.

Antiviral activity. Inhibition of virus-induced cytopathogenicity was measured following well-established procedures (6, 10). In all viral cytopathogenicity assays, the virus inoculum was 100 $CCID_{50}$s (1 $CCID_{50}$ corresponds to the virus stock dilution that proved infective for 50% of the cell cultures) per microtiter well. In the CMV assays, in which plaque formation instead of viral cytopathogenicity was measured, the virus inoculum was 20 PFU.

Vaccinia virus yield reduction experiments were carried out in L-929 cells. The cell monolayers were infected with vaccinia virus at $10^{4.5}$ PFU/0.5 ml per petri dish. After 1 h of virus adsorption, residual virus was removed and the cells were incubated further in the presence of various concentrations of the test compounds. Virus yield was measured at 24 and 48 h after virus infection. To this end, the cell cultures were frozen at −70°C and thawed, and the cell homogenates were assayed for virus content by plaque formation in L-929 cells.

Antimetabolic activity. Inhibition of host cell macromolecule (DNA, RNA, and protein) synthesis was monitored by incorporation of [*methyl*-^{3}H]dThd, [5-^{3}H]Urd, and [4,5-^{3}H] Leu, respectively, over an incubation period of 16 h of exponentially growing (PRK, Vero, or HeLa) cells in the presence of various concentrations of the test compounds.

Cytostatic activity. Inhibition of the proliferation of PRK, Vero, HeLa, HEL, and MT-4 cells was assessed during their exponential growth phase and was monitored by counting the number of viable cells (following staining with trypan blue). The procedure was similar to that initially described for L1210 cells (9).

***S*-Adenosylhomocysteine hydrolase activity.** The inhibitory effects of the test compounds on *S*-adenosylhomocysteine (SAH) hydrolase, purified from L-929 cells, were measured as described previously (5).

Pox tail lesion model. NMRI mice (weight, 11 to 13 g) were inoculated intravenously (in a tail vein) with 0.2 ml of a virus dilution containing approximately 5×10^4 PFU/ml. The mice were treated for 5 days, starting 1 h after virus infection, with the test compounds administered intraperitoneally at a dosage of 5, 10, or 25 mg/kg/day. Tail lesions were enumerated 7 days after infection. The statistical significance of the differences in the numbers of lesions between the treated and control mice was assessed by Student's *t* test.

RESULTS

Antiviral spectrum and activity. The 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides were evaluated in a panel of virus assay systems similar to that originally used to assess the antiviral effects of FICAR and ribavirin (11). Marked activity was noted against vaccinia virus in PRK cells; VSV in HeLa (but not in PRK) cells; coxsackievirus type B4 in HeLa cells; and parainfluenza virus type 3, reovirus type 1, Sindbis virus, and Semliki forest virus in Vero cells. EICAR proved effective against the ortho- and paramyxoviruses, influenza virus types A and B, parainfluenza virus type 3, measles virus, subacute sclerosing panencephalitis virus, and RSV at a concentration that was 10- to 30-fold lower than that of ribavirin. EICAR also proved active against the arenaviruses Junin and Tacaribe in Vero cells (50% inhibitory concentration [IC_{50}], 0.2 μg/ml) (Table 1).

Although the antiviral activity of EICAR and its congeners varied considerably from one system to another, their activity spectra were remarkably similar to that of ribavirin. Also, the relative order of antiviral potency remained fairly constant throughout the different assay systems; thus, the potencies were as follows: EICAR (compound 1) > compound 2 > compound 3 > ribavirin > compound 4 > compound 5. The antiviral potency shown by EICAR was about 10- to 100-fold greater than that of ribavirin. Under the conditions used (resting confluent cells) for measuring the antiviral effects of EICAR and ribavirin, they did not prove to be toxic to the host cells at the highest concentration tested (400 μg/ml).

EICAR inhibited the replication of CMV (strains AD-169 and Davis) in HEL cell monolayers at an IC_{50} of 0.4 to 0.5 μg/ml and the proliferation of exponentially growing uninfected HEL cells at an IC_{50} of 2 μg/ml. Ribavirin did not inhibit CMV replication up to a concentraton of 400 μg/ml, while it inhibited exponential growth of HEL cells at an IC_{50} of 20 μg/ml. EICAR was not inhibitory to human immunodeficiency virus type 1 replication at concentrations that were not toxic to growing MT-4 cells (IC_{50}, 0.36 μM). Similarly, ribavirin was not effective against human immu-

TABLE 1. Inhibitory effects of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides on virus replication in various assay systems

Virus	Cell	IC_{50} (μg/ml)[a]						
		Compound 1 (EICAR)	Compound 2	Compound 3	Compound 4	Compound 5	C-c^{3}Ado	Ribavirin
Herpes simplex type 1	PRK	>400	>400	>400	>400	>400	>400	>400
Herpes simplex type 2	PRK	>400	>400	>400	>400	>400	>400	>400
Vaccinia	PRK	0.2	1	2	150	300	2	20
Vesicular stomatitis	PRK	>400	>400	>400	>400	>400	2	>400
Vesicular stomatitis	HeLa	4	10	20	>200	>400	2	20
Coxsackie type B4	HeLa	2	10	>200	>200	>200	>400	40
Polio type 1	HeLa	>400	>400	>200	>200	>400	>400	>400
Respiratory syncytial	HeLa	0.2	ND[b]	ND	ND	ND	ND	6
Parainfluenza type 3	Vero	2	4	7	>200	>400	4	40
Measles	Vero	0.5	ND	ND	ND	ND	ND	8
SSPE[c]	Vero	0.3	ND	ND	ND	ND	ND	10
Reo type 1	Vero	1	4	7	70	>200	1	100
Sindbis	Vero	2	10	20	300	>400	20	70
Semliki forest	Vero	2	10	20	>400	>400	>400	>400
Influenza A	MDCK	0.9	ND	ND	ND	ND	ND	8
Influenza B	MDCK	1	ND	ND	ND	ND	ND	9

[a] IC_{50}, 50% inhibitory concentration, or concentration required to inhibit viral cytopathogenicity by 50%. No microscopically visible alteration of normal cell morphology or reduction in cell viability (as assessed by trypan blue exclusion) was noted for any compound at the highest concentration tested (400 μg/ml). The data represent mean values for two to four separate experiments.

[b] ND, Not determined.

[c] SSPE, Subacute sclerosing panencephalitis virus. Identical results were obtained for the Niigata-1 and Kitaken-1 strains.

nodeficiency virus type 1 replication at 10 μM, the highest concentration not toxic to MT-4 cells (see also reference 2).

EICAR, compound 2, and ribavirin effected a concentration-dependent reduction of vaccinia virus yield in L-929 cells, whether virus yield was determined at 24 or 48 h after infection (data not shown). The concentrations to reduce virus yields by 90% at 24 h after infection were 5 μM for EICAR, 17 μM for compound 2, and 240 μM for ribavirin. These values confirmed the relative order of antiviral potencies (EICAR > compound 2 > ribavirin) noted above.

Mechanisms of antiviral action. EICAR was active against VSV in HeLa cells but not in PRK cells (Table 1). To delineate the cell dependence in the antiviral activity, EICAR, compound 2, ribavirin, and C-c^{3}Ado were further examined for their activity against VSV in a wide variety of cell lines. EICAR was active against VSV in HeLa, Vero, E_6SM, and BSC-1A cells (IC_{50}s, 1 to 10 μg/ml) but not in other cell lines (PRK, HEL, RK-13, GBK, CV-1, and L-929 cells) (IC_{50}, >200 μg/ml). Remarkably, compound 2 and ribavirin showed the same cell dependence pattern, whereas C-c^{3}Ado did not. C-c^{3}Ado was active (IC_{50}, 2 μg/ml) against VSV in PRK, RK-13, GBK, and L-929 cells (i.e., cell lines in which EICAR did not prove active against VSV), but C-c^{3}Ado was inactive against VSV in BSC-1A cells (a cell line in which EICAR inhibited the cytopathogenicity of VSV).

The dissimilarities in the antiviral activity spectra of C-c^{3}Ado, a carbocyclic adenosine analog whose antiviral activity depends on the inhibition of SAH hydrolase (7), and EICAR (Table 1) as well as the differences in the cell dependence of their antiviral activities indicate that these two compounds differ in their mechanisms of action. When compound 1 (EICAR) and compound 2 were evaluated for their inhibitory effects on SAH hydrolase (purified from L-929 cells), neither compound 1 nor 2 proved inhibitory to the enzyme at a concentration of 100 μM. Similarly, ribavirin failed to inhibit SAH hydrolase activity at a concentration of 100 μM. In marked contrast, C-c^{3}Ado achieved 50% inhibition of SAH hydrolase activity at a concentration of 0.018 μM (5).

The antiviral effects of EICAR and ribavirin against VSV, parainfluenza virus type 3, reovirus type 1, and Sindbis virus were virtually abolished on the addition of guanosine at 10 μg/ml (Table 2). At 100 μg/ml, guanosine also abolished the activity of EICAR against RSV, but at this concentration guanosine increased the activities of EICAR and ribavirin against some of the other viruses (i.e., parainfluenza virus type 3 and reovirus type 1). In the presence of guanosine at 100 μg/ml, which, by itself, was not inhibitory to viral cytopathogenicity, the IC_{50} of EICAR for parainfluenza virus type 3 and reovirus type 1 in Vero cells decreased from 1–2 to 0.02–0.1 μg/ml (data not shown). Addition of any of the other nucleosides (adenosine, inosine, cytidine, or uridine) at either 10 or 100 μg/ml (Table 2) (data not shown) neither diminished nor potentiated the activity of EICAR or ribavirin against any of the viruses tested.

Cytostatic activity. The antimetabolic effects of EICAR and its congeners were examined in (exponentially) growing PRK, Vero, and HeLa cells. EICAR and the other 5-alkynylimidazole derivatives were found to inhibit host cell DNA and RNA synthesis but not protein synthesis (Table 3). The IC_{50} of EICAR for cellular DNA synthesis was 0.48 μg/ml (PRK cells), 1.8 μg/ml (Vero cells), and 0.32 μg/ml (HeLa cells). The corresponding IC_{50}s for RNA synthesis were 1, 1.1, and 1.25 μg/ml, respectively. When DNA synthesis (in PRK cells) was monitored with $^{32}P_i$, instead of [*methyl*-^{3}H]dThd, incorporation, similar IC_{50}s were obtained (1.8 ± 1.0 μg/ml for EICAR and 3.5 ± 2.0 μg/ml for compound 2). This indicates that under the experimental conditions used, [*methyl*-^{3}H]dThd incorporation was as good a measure of DNA synthesis as $^{32}P_i$ incorporation was (13).

When EICAR was examined for its antiproliferative ac-

TABLE 2. Reversal of antiviral effects of EICAR (compound 1) and ribavirin following addition of different nucleosides

Compound and virus	Cell	IC_{50} (μg/ml)[a] of EICAR or ribavirin following the addition of:					
		No nucleoside	Guanosine	Adenosine	Inosine	Cytidine	Uridine
EICAR							
RSV	HeLa	0.7	>200	0.7	0.7	0.7	0.7
Vesicular stomatitis	HeLa	1	>400	2	2	0.7	2
Parainfluenza type 3	Vero	1	100	1	1	1	0.7
Reo type 1	Vero	2	>200	2	2	2	2
Sindbis	Vero	4	>400	2	4	2	2
Ribavirin							
RSV	HeLa	8	90	8	8	4	4
Vesicular stomatitis	HeLa	20	100	20	20	20	20
Parainfluenza type 3	Vero	40	100	40	40	20	40
Reo type 1	Vero	150	>400	150	150	200	100
Sindbis	Vero	70	>400	100	70	70	100

[a] IC_{50}, 50% inhibitory concentration, or concentration required to inhibit viral cytopathogenicity by 50% (mean values for two to four separate experiments). The nucleosides were added at 10 μg/ml, except for the RSV experiments, in which they were added at 100 μg/ml. At the concentrations used, the nucleosides had no direct effect on viral cytopathogenicity.

tion, it proved inhibitory to the growth of PRK, Vero, and HeLa cells at IC_{50}s ranging from 0.21 to 0.74 μg/ml (Table 4). Ribavirin was also cytostatic to these cells, but only at concentrations which were about 15- to 30-fold higher than the concentrations at which EICAR inhibited cell growth. In contrast, tubercidin, a well-known cytotoxic agent, inhibited cell growth at a concentration which was 100-fold lower than the IC_{50} of EICAR.

In vivo anti-vaccinia virus activity. When administered daily at a dose of either 10 or 25 mg/kg, EICAR effected a significant reduction in the number of vaccinia virus tail lesions, without apparent toxicity for the host (Table 5). Partial suppression of vaccinia virus tail lesion formation was also observed with ribavirin when it was administered at a dosage of 25 mg/kg/day. The latter is in accord with previous observations in the same animal model (12).

DISCUSSION

EICAR can be considered a structurally related analog of ribavirin, in which the N at position 2 of the triazole ring has been replaced by a C—C≡CH group. The antiviral activity spectrum of EICAR is similar to that of ribavirin. Yet, EICAR is more potent than ribavirin. EICAR has proved to be particularly active against poxviruses (vaccinia virus), togaviruses (Sindbis and Semliki forest viruses), arenaviruses (Junin and Tacaribe viruses), reoviruses (reovirus type 1), orthomyxoviruses (influenza virus types A and B), and paramyxoviruses (parainfluenza virus type 3, measles virus SSPE virus, and RSV). The antiviral activity of EICAR is cell dependent. A similar cell dependence was observed for the antiviral activity of ribavirin. This is in keeping with previous studies (18), in which the activity of ribavirin against several viruses (i.e., herpes simplex virus, vaccinia virus, and VSV) was found to vary considerably according to the cell line used.

EICAR was originally recognized as an antileukemic agent (25). In fact, EICAR is a more potent inhibitor of cell proliferation than ribavirin is (Table 4). It also inhibits DNA and RNA synthesis of exponentially growing cells at a concentration which, on the one hand, is lower than the concentration at which ribavirin inhibits cellular DNA and RNA synthesis (Table 3) and, on the other hand, corresponds closely to its cell growth-inhibitory concentration.

From our data, EICAR does not appear to have a selectivity (as an antiviral agent) greater than that of ribavirin. It may have potential as both an antiviral and cytostatic agent, because these properties are achieved under different conditions, and thus are not mutually exclusive. Antiviral activity was demonstrated in resting cells, whereas cytostatic activity was established in exponentially growing cells. Under the conditions used for the antiviral activity measurements, EICAR did not show toxicity to the host cells.

TABLE 3. Inhibitory effects of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides on cellular DNA, RNA, and protein synthesis in exponentially dividing cells

Synthesis[a]	Cell	IC_{50} (μg/ml)[b]						
		Compound 1 (EICAR)	Compound 2	Compound 3	Compound 4	Compound 5	C-c^{3}Ado	Ribavirin
DNA	PRK	0.48 ± 0.4	1.0 ± 0.5	8.9	>50	19.3	>200	5.5 ± 2.9
RNA	PRK	1 ± 0.7	1.7 ± 0.3	32.5	>50	21.2	98.5	11.5 ± 5
Protein	PRK	>200	>200	>200	>50	>50	>200	>200
DNA	Vero	1.8 ± 1.5	3.3 ± 2.1	4.8	>50	23	>200	8.3 ± 2.5
RNA	Vero	1.1 ± 0.3	4.3 ± 2.7	5.4	>50	7.8	138	28.7 ± 12
Protein	Vero	>200	>200	>200	>50	>50	>200	>200

[a] DNA, RNA, and protein synthesis was measured by the incorporation of [*methyl*-^{3}H]dThd, [5-^{3}H]Urd, and [4,5-^{3}H]Leu, respectively.
[b] IC_{50}, 50% inhibitory concentration, required to inhibit DNA, RNA, or protein synthesis by 50%. Values are means ± standard deviations for three separate experiments.

TABLE 4. Inhibitory effects of EICAR (compound 1), compound 2, C-c^{3}Ado, ribavirin, and tubercidin on cell proliferation

Cell	IC_{50} (μg/ml)[a]				
	Compound 1 (EICAR)	Compound 2	C-c^{3}Ado	Ribavirin	Tubercidin
PRK	0.37 ± 0.21	0.55 ± 0.22	2.2 ± 1.7	5.6 ± 1.3	0.0061 ± 0.004
Vero	0.74 ± 0.23	0.61 ± 0.33	11 ± 2	25 ± 8	0.0063 ± 0.0007
HeLa	0.21 ± 0.04	ND[b]	2.0 ± 1.3	7.1 ± 3.7	0.0065 ± 0.003

[a] IC_{50}, 50% inhibitory concentration, or concentration required to inhibit cell proliferation by 50%. Values are means ± standard deviations for three separate experiments.
[b] ND, Not determined.

From the antiviral activity spectrum of EICAR, it can be inferred that its mode of action is similar to that of ribavirin. Although the primary site for the antiviral action of ribavirin has remained a point of some controversy, it has been originally identified as the enzyme IMP dehydrogenase that converts IMP to XMP (38). Consequently, GMP, GDP, and GTP pool levels are reduced and viral RNA synthesis is suppressed. The fact that the antiviral effects of ribavirin are readily reversed following the exogenous addition of guanosine (4, 31) indicates that ribavirin may indeed act via depletion of the GTP pool. This does not exlude, however, the possibility that under certain conditions ribavirin may also act as an inhibitor of viral mRNA capping (16) or as an inhibitor of primer generation and elongation during influenza viral RNA transcription (41).

When added to the cells at the appropriate concentration (i.e., 10 μg/ml), guanosine reversed not only the antiviral activity of ribavirin but also that of EICAR, whereas other nucleosides (i.e., adenosine, inosine, cytidine, and uridine) failed to do so (Table 4). This adds further support to the hypothesis that both EICAR and ribavirin act via depletion of the GTP pool and may be targeted at IMP dehydrogenase (38).

Inhibition of IMP dehydrogenase is also believed to account for the antitumor activity of ribavirin (24), tiazofurin (19), and selenazofurin (3); and likewise, IMP dehydrogenase may be postulated as a target for the cytostatic action of EICAR and its congeners. That the same enzyme may serve as a target for both the antiviral and anticellular (antitumor) actions of the same compound is not surprising since virus-infected cells and rapidly growing (tumor) cells may impose similar requirements on host cell metabolism, particularly with regard to the supply of ribo- and deoxyribonucleotides for their RNA and DNA synthesis. It is now mandatory to assess directly the role of IMP dehydrogenase as a target enzyme for EICAR 5′-monophosphate.

EICAR is capable of achieving in vivo activity, as demonstrated in the pox tail lesion model (Table 5). EICAR should be further evaluated for its efficacy in other model infections with those viruses that are particularly susceptible to the compounds (i.e., influenza virus types A and B and RSV). The fact that EICAR is about 10- to 30-fold more potent in vitro than ribavirin against these viruses makes it an attractive candidate drug for the treatment of orthomyxo- and paramyxovirus infections.

TABLE 5. Comparative inhibitory effects of EICAR (compound 1) and ribavirin on the formation of vaccinia tail lesions in mice[a]

Compound	Daily dose (mg/kg)	No. of lesions/mouse (mean ± SD)	P^b
EICAR	25	9.2 ± 8.0	<0.005
	10	18.7 ± 6.8	<0.005
	5	30.0 ± 6.5	NS
Ribavirin	25	22.7 ± 8.1	<0.025
	10	29.8 ± 6.8	NS
	5	25.6 ± 8.4	NS
Control		29.6 ± 6.6	

[a] Vaccinia virus was injected intravenously. The compounds were administered intraperitoneally, starting immediately after virus inoculation, and administration was continued daily for 5 consecutive days. There were 10 mice in each dosage group.
[b] Statistical significance was assessed by Student's *t* test. NS, Not significant.

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