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NOTES

In Vitro Effect of Virazole Against Influenza Viruses

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The minimal inhibitory concentrations of Virazole against 32 mean tissue culture infective doses of three type A influenza strains including type A/England/42/72 (H3N2) and a type B strain in tissue culture were 0.1 and 0.05 μg/ml, respectively. The growth inhibition pattern by various Virazole concentrations of type A virus was similar to that of the type B virus. Virazole appears to be slightly more potent against the A/England/42/72 strain than are other antiinfluenzal agents.

Virazole (ICN 1229, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamine) has been reported as a broad-spectrum antiviral agent effective against both deoxyribonucleic acid and ribonucleic acid viruses, including type A (A2/Japan/305/57 and A2/Aichi/2/68) and B (B/Lee/40) influenza viruses (2, 4). The antiinfluenzal activity of this compound was investigated in primary rhesus monkey kidney cell cultures (RMKs). The viruses were two strains of the most recent variants of type A virus (H3N2), A/University of Maryland/2/72 (an isolate from the influenza outbreak in Baltimore in the winter of 1972, passage level—RMK1.Egg2.RMK1) and a/England/42/72 (RMK 1.E9.RMK1), A/Maryland/104/68 (a A/Hong Kong/68 strain—RMK4), and B/University of Maryland/1/71 (RMK4). Three other antiinfluenzal compounds, adamantane hydrochloride (3), cyclooctylamine hydrochloride (Smith Kline & French 23880A) (1), and DU34796 [1'-methyl spiro (amantadine 2,3'-pyrrolidine) maleate (1:1)] (A. Peters et al., Proc. Int. Congr. Chemother, 7th, 1970, **2**:71; N. V. Philips-Duphar, personal communication) were tested simultaneously. RMKs were maintained in Eagle basal medium with Earle balanced salt solution. RMKs in tubes were used, and they were incubated at a stationary position at 35 C. The influenza virus growth in RMKs was determined by the hemadsorption technique.

In the experiment in which the minimal inhibitory concentration (MIC) of these drugs against influenza strains was evaluated, drug concentrations ranging from 25 to 0.01 μg/ml were tested against 32 mean tissue culture infective dose ($TCID_{50}$) of the virus. A twofold dilution of the drug was added to each of two RMKs which were then incubated at room temperature for 1 h, after which the virus fluid was added to make a final viral dose of 32 $TCID_{50}$ in the medium. The medium was not changed during the test period of 3 days. In another experiment, where the reduction of the virus titers by various concentrations of the drug was determined, each of the virus strains,

TABLE 1. *Minimal inhibitory concentrations of Virazole and other antiinfluenzal compounds against type A and B influenza strains in RMK cell cultures*[a]

Compound	A/University of Maryland/2/72	A/England/42/72	A/Maryland/104/68	B/University of Maryland/1/71
Virazole	0.1	0.1	0.1	0.05
Amantadine hydrochloride	0.05	0.05	0.05	>25
Cyclooctylamine hydrochloride	0.1	0.05	0.1	>25
DU34796[b]	0.1	0.1	0.1	>25

[a] Concentration values are expressed in micrograms per milliliter versus 32 $TCID_{50}$.

[b] 1'-Methyl spiro (adamantane 2,3'-pyrrolidine) maleate (1:1).

TABLE 2. *Reduction of type A and B influenza virus titers[a] in RMK cell cultures in the presence of Virazole and amantadine hydrochloride*

Compound (μg/ml)	A/University of Maryland/2/72 (day postinoculation)						B/University of Maryland/1/71 (day postinoculation)					
	1	2	3	4	5	6	1	2	3	4	5	6
Virazole												
25	0[b]	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
1.0	0	2.5	4.5	3.0	1.5	1.5	1.0	1.0	2.5	2.0	1.5	1.5
0.1	0	3.5	5.5	5.0	3.5	1.5	1.5	4.0	6.5	5.0	4.5	1.5
0	2.5	4.5	6.5	6.5	5.5	4.0	1.5	3.5	6.5	6.5	5.5	4.5
Amantadine hydrochloride												
25	0	0	0	0	0	0	1.5	1.5	4.5	4.5	5.5	4.0
10	0	1.0	3.5	3.5	2.0	2.0	1.5	2.5	6.5	5.5	5.5	4.5
1.0	0	3.5	5.0	6.0	5.5	4.0	1.5	3.5	6.5	5.5	5.0	4.5
0.1	2.0	5.0	6.5	6.5	5.5	5.5	1.5	3.0	6.5	5.5	5.5	4.5
0	2.5	4.5	6.5	6.5	5.5	4.0	1.5	3.5	6.5	6.5	5.5	4.5

[a] Log_{10} $TCID_{50}$/ml.
[b] 0, Virus not detected.

A/University of Maryland/2/72 (10^5 $TCID_{50}$) and B/University of Maryland/1/71 ($10^{5.5}$ $TCID_{50}$), was inoculated into four sets of eight RMK 1 h after the addition of 25, 10, 1.0, and 0.1 μg of the drug per ml to each set. The virus control RMK had medium without drug. During a 6-day test period, the medium was harvested once a day and was replaced with fresh medium with added drug. The medium from eight RMKs was pooled and stored at −60 C. The virus titer in each of the harvests was determined in RMK. Each of two RMKs was inoculated with a 10-fold dilution of the fluid and was incubated for 3 days. The titration of virus in all harvests from the type A virus study was carried out simultaneously in one experiment, as in the type B virus study.

The MIC of the 4 drugs against 32 $TCID_{50}$ of all strains of type A influenza virus was similar, i.e., 0.1 or 0.05 μg/ml (Table 1). The MIC against 320 $TCID_{50}$ of the A/Maryland/104/68 was determined, and two- to eightfold increases in concentration of these drugs was observed. The type B strain was as sensitive as type A strains to Virazole, but was resistant to 25 μg/ml of the other three drugs.

The influenza growth in the presence of four different concentrations of Virazole and amantadine is shown in Table 2. The A/University of Maryland/2/72 was not detected in RMK which received 25 and 10 μg of Virazole and 25 μg of amantadine per ml. Partial, but significant, growth inhibition was noted at 1.0 μg of Virazole and 10 μg of amantadine per ml. The virus titers in 0.1 μg of Virazole and 1.0 and 0.1 μg of amantadine per ml were not different from those of the virus control, except on day 1 when the virus was not detected in the drug-treated RMK. The B/University of Maryland/1/71 growth was suppressed completely at 25 and 10 μg of Virazole per ml and markedly at 1.0 μg/ml. The virus was not affected at 0.1 μg/ml. No inhibition of this virus by amantadine was apparent. The growth patterns of type A and B influenza viruses in cyclooctylamine and DU34796 were essentially the same as those in amantadine. There was no evidence of cytotoxic effects to RMK with 25 μg/ml of any of the four drugs during the test period.

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