References and Notes

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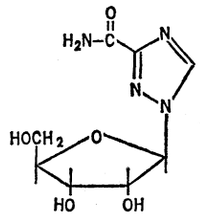
30 March 1972; revised 5 June 1972

Broad-Spectrum Antiviral Activity of Virazole: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide

Abstract. *Virazole is a synthetic nucleoside active in tissue culture against at least 16 DNA and RNA viruses. Applied topically, it inhibits herpetic keratitis in rabbits and tail lesions induced by herpes, vaccinia, and vesicular stomatitis viruses in mice. Injected intraperitoneally into mice, it inhibits splenomegaly and hepatomegaly induced by Friend leukemia virus and respiratory infections caused by influenza A_0, A_2, and B viruses and parainfluenza 1 virus. Oral or aerosol treatment of parainfluenza virus infections is also effective.*

Few chemicals have been reported which have broad-spectrum antiviral activity, and the majority of these are inducers of interferon, hence effective primarily as prophylactic rather than therapeutic agents. No synthetic compounds are known which have significant in vitro and in vivo antiviral activity against both DNA and RNA viruses. We describe the broad-spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, ICN-1229), a water-soluble, stable, colorless nucleoside, the synthesis of which has been reported (*1*).



Virazole

In tissue culture experiments, inhibition of virus-induced cytopathogenic effect was determined in the appropriate cell lines grown in disposable plastic microplates as described (*2*). Inhibition of cytopathogenic effect was evaluated by the virus rating method (*3*). Inhibition of virus production, as measured by extra- and intracellular virus titers (*2*), was also studied. Because the influenza viruses produced little discernible cytopathogenic effect in the cells used, reduction in supernatant hemagglutination titers, expressed as therapeutic index (*4*), was used to determine antiviral effect. As judged by these measurements, Virazole was markedly inhibitory to both RNA and DNA viruses (Table 1). Drugs known to be active against one or more of the viruses listed in Table 1 were tested against those viruses in experiments run parallel to those with Virazole. In most of these comparative evaluations, Virazole had antiviral activity equal to, or greater than, the known active compound (*5*), and its spectrum of activity exceeded that of all of the compounds studied.

To determine if Virazole induced interferon, we evaluated the chemical both in vitro (*2*) and in vivo. Virazole-free medium collected 24 or 48 hours after a 3-hour incubation of L-929 cells with the compound (1000 μg/ml) contained no interferon-like activity against vesicular stomatitis virus. The cells in this experiment were not rendered refractory to infection. In the in vivo system, male Swiss-Webster mice (18 to 22 g) were injected once intraperitoneally with Virazole (1000 mg per kilogram of body weight), and serums collected from these animals 2 to 24 hours later were assayed for interferon activity in L-929 cells. No substance inhibitory to vesicular stomatitis virus was found in these serums, a result indicating that Virazole was not an inducer of interferon. The compound, tested as described (*2*), was not virucidal against herpes simplex or parainfluenza virus.

The following animal experiments were designed to determine if the broad in vitro antiviral activity would also be seen in vivo. The corneal epithelia of both eyes of New Zealand albino rabbits (3 to 5 kg) were uniformly scratched, and two drops of a suspension containing herpes simplex virus (McKrae) of known titer were instilled in each. One eye of each rabbit was treated with Virazole for 7 days. Virazole (10, 1, or 0.1 percent), dissolved in 1.4 percent polyvinyl alcohol, was applied hourly from 8 a.m. to 7 p.m., and at 8 p.m. eyes were treated with Virazole suspended in Jellene base ophthalmic ointment containing 1 percent chloramphenicol (Parke-Davis, Detroit). Treatment began 4 hours after virus inoculation. The remaining eye of each rabbit was similarly treated with polyvinyl alcohol and ointment devoid of Virazole to serve as control. Each eye was examined daily, both grossly and by biomicroscope, on a blind basis for infectivity (lesion size and type, corneal opacity) and for inflammatory response (erythema, chemosis, discharge), and the weighted grading scale described by Corwin *et al.* (*6*) was used. The eyes treated with 10 or 1 percent Virazole showed significant improvement ($P < .01$, as determined by ranking analysis) over placebo-treated eyes. This improvement was evident from both the weighted scale and from each individual scoring method. Uninfected, treated controls examined in parallel with infected animals revealed no toxic effects in any of the treated eyes.

Virazole was evaluated in the system of virus-induced mouse tail lesions described by Yoshimura *et al.* (*7*). A 20 percent solution of the compound in polyvinyl alcohol, applied topically to the tail twice daily for 15 days (the first application 18 hours after virus inoculation), inhibited the development of the lesions induced by herpes simplex, vaccinia, and vesicular stomatitis viruses. The probability values (*8*) for this lesion inhibition were $< .01$ for the period between 5 and 30 days after virus inoculation. No skin irritation was seen in uninfected, treated animals.

Splenomegaly and hepatomegaly induced by Friend leukemia virus (*9*) was significantly inhibited by intraperitoneal treatment with Virazole (100 mg/kg) administered twice daily for 9 days, with the first injection 4 hours before virus inoculation. Total body weights, as well as spleen and liver weights, were determined on day 14;

Table 1. In vitro antiviral activity of Virazole. In all experiments, Virazole was added 15 to 30 minutes after cultures were inoculated with ~100 times the virus dose needed to infect 50 percent of cells (~100 $CCID_{50}$). Virus abbreviations: HS, herpes simplex; PR, pseudorabies; MCM, murine cytomegalovirus; AV, adenovirus; PI, parainfluenza; RV, rhinovirus; PV, poliovirus; VS, vesicular stomatitis; SF, Semliki Forest. Cell abbreviations: KB, human carcinoma of the nasopharynx; RK-13, continuous rabbit kidney; ME, primary mouse embryo; CE, primary chick embryo; L-929, mouse fibroblast.

Virus	Cell line	Virus rating
DNA viruses		
HS type 1 (HF)	KB	1.2*
HS type 2 (MS)	KB	1.1
PR (RK17C24, derived from Aujeszky)	RK-13	0.0†
MCM (Smith)	ME	0.9
Vaccinia (Lederle CA)	KB	1.0*
Myxoma (Sanarelli)	RK-13	1.7
AV type 3 (GB)	KB	0.7
RNA viruses		
PI type 3 HA-1 (C243)	KB	0.8*
PI type 1 (Sendai)	CE	≧ 10‡
Influenza A_2 (Jap/305)	CE	3.2‡
Influenza B (Lee)	CE	10‡
RV type 1A (2060)	KB	0.6
RV type 13 (353)	KB	0.8
RV type 56 (Phillips)	KB	0.7
Coxsackie B_1 (Conn.-5)	KB	0.4*
PV type 2 (MEF-1)	KB	0.0†
VS (Indiana)	KB	0.7*
SF (original)	L-929	0.6

* Intra- and extracellular virus titers were also determined with these viruses in the presence of Virazole; significant (> 1.0 $\log_{10}$) reductions were seen. † Virus ratings of as high as 0.8 were obtained against these viruses if the cells were first exposed to Virazole. ‡Therapeutic indices were determined for these viruses.

animal weight loss was negligible. Intraperitoneal injections of Virazole (250 mg/kg), given to similarly infected mice 4 hours after virus inoculation and again on day 7, also resulted in a significant decrease in the splenomegaly on day 14, although the liver weight was not reduced. The body weight in treated and control groups was virtually the same on day 14. Intracerebral infections with herpes simplex, vaccinia (WR), Semliki Forest, or Western equine encephalitis (M28) viruses in Swiss-Webster mice were not significantly altered by intraperitoneal or intravenous treatment with Virazole, which suggests that the drug may have difficulty in crossing the blood-brain barrier.

Virazole appeared to be effective against respiratory infections by RNA viruses. Swiss mice (13 to 15 g) infected intranasally with influenza A_2, A_0, or B viruses were treated intraperitoneally twice daily for 7 days with Virazole dissolved in saline. Treatment began 4 hours before virus inoculation. Survivor increases of as much as 70 percent, accompanied by significant increases in mean survival time of dying animals, were seen in the treated animals as compared to controls during a 21-day period (Table 2). In mice infected with parainfluenza 1 virus by aerosol (*10*) and similarly treated with Virazole, the number of survivors increased as much as 90 percent (Table 2). In other experiments (*11*), Virazole administered orally or by aerosol was effective against parainfluenza 1 virus infections, and intraperitoneal treatment as late as 96 hours after virus inoculation significantly inhibited the course of the infection.

Initial experiments indicate that Virazole inhibits DNA synthesis in both infected and uninfected RK-13 and KB cells, as determined by [^{3}H]-thymidine uptake, although this inhibition was not more pronounced than that observed with 1-β-D-arabinofuranosylcytosine, a drug known (*12*) to be effective against DNA viruses. Virazole also seems to partially inhibit RNA synthesis, although it has little apparent effect on protein synthesis in these same cells (*11*). Such possibly toxic manifestations, although important, would appear to be overshadowed by the survival and apparent health of large numbers of Virazole-treated animals infected with often lethal doses of virus. For Virazole given in one dose, the lethal dose for 50 percent of mice (LD_{50}) was approximately 2000 mg/kg given orally or 1200 mg/kg given intraperitoneally. When the drug was given twice daily for 9 days by either route, the LD_{50} was approximately 250 mg per kilogram per day.

The antiviral spectrum for Virazole thus appears to be the broadest ever reported for a synthetic material that does not induce interferon.

Table 2. Activity of Virazole against lethal respiratory virus infections (10 to 20 LD_{50}) in mice. Deaths were recorded for 21 days, and survivors were considered to have died on day 21 in determining mean day of death. Groups of 10 to 20 mice were used in each experiment (T, treated; C, control).

Virazole (mg/kg per day)	Survivors (%)		P*	Day of death (mean)		P†
	T	C		T	C	
Influenza A_0 (PR8)						
75	90	20	< .001	20.1	10.2	< .001
37.5	40	20	> .3	12.4	10.2	< .05
Influenza A_2 (Jap/305)						
150	100	30	< .001	> 21.0	10.0	< .001
75	70	30	< .001	16.8	10.0	< .001
Influenza B (Lee)						
30	40	0	< .05	16.0	8.4	< .001
15	20	0	> .3	12.2	8.4	< .05
Parainfluenza 1 (Sendai)						
75	90	0	< .001	19.9	9.8	< .001
37.5	70	0	< .001	18.3	9.8	< .001

* Probability value (chi-square analysis). † Probability value (Student's *t*-test).

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4. The therapeutic index for a chemical is the ratio of the highest nontoxic concentration to the lowest effective concentration. In these studies, toxicity was determined by microscopic examination of drug-exposed cells. An effective concentration was one for which the average HA titer was less than the average virus-control titer by a factor of 2.
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13 March 1972