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Effect of Isoprinosine Against Influenza and Some Other Viruses Causing Respiratory Diseases

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The antiviral activity of isoprinosine was tested in tissue cultures and mice. In tissue cultures, concentrations of 25 to 100 μg/ml inhibited the infectivity of influenza and herpes hominis viruses but not parainfluenza virus, rhinovirus, or adenovirus. Among different strains of influenza A, there was considerable variability in the inhibitory concentration of isoprinosine. For influenza B, a zone effect was observed in the inhibitory drug concentration. Oral prophylactic administration of isoprinosine beginning 24 hr before infection with an intermediate challenge dose of influenza A and continued as treatment for 5 days produced a significant reduction in mortality. No protection was provided against a high dose challenge. Oral or intraperitoneal treatment of mice beginning 24 hr after infection with influenza A or B viruses significantly delayed or prevented death when the drug was administered for 10 days, but not when treatment was limited to 4 days. An increased fatality rate which occurred in treated mice given a virus dose of low lethality could not be attributed to drug toxicity.

Isoprinosine (NPT 10381, NP-113) is the paracetamidobenzoic acid salt of inosine dimethylaminoisopropanol in a 1:3 molar ratio (1). In preliminary communications, it was reported that the drug inhibited certain viruses causing respiratory disease in man (E. R. Brown and P. Gordon, Proc. Fed. Amer. Soc. Exp. Biol. **30**:242, 1971; P. Gordon, E. R. Brown, and B. Ronsen, Proc. Fed. Amer. Soc. Exp. Biol. **30**:242, 1971). Potential advantages of isoprinosine are that it is effective when administered orally and that it can exert a therapeutic effect when administered after the establishment of infection. To provide further experience and gain better definition of the antiviral activity of isoprinosine, investigations were performed in tissue cultures and in mice. Both prophylactic and therapeutic use and oral and parenteral administration of the drug were studied in mice infected with influenza A/Beth/10/63 (H2N2) or influenza B/Maryland/1/59.

MATERIALS AND METHODS

Drug. Isoprinosine, lot AA2061, was furnished by Newport Pharmaceuticals, Newport Beach, Calif. The concentrations used in tissue culture studies ranged from 10 to 100 μg/ml. In mice, 0.4 to 1.2 g per kg per day was given in water solution.

Tissue culture. Primary rhesus monkey kidney cell cultures in tubes maintained with medium 199, containing 1% anti-simian virus 5 rabbit serum, were used to test drug efficacy against myxoviruses, paramyxoviruses, and herpes hominis virus. WI-38 cells, maintained with medium 199 containing 2% fetal calf serum, were used for testing the effect on rhinoviruses and adenoviruses. To measure drug inhibition, three to five tubes per dilution were used in a checkerboard with six dilutions of drug and five to six log dilutions of virus.

Mice. Under light ether anesthesia (by inhalation), female mice (12 to 14 g) of strain CD-1 (Chas. River, N. Wilmington, Mass.) were challenged with virus given as nose drops in a volume of 0.05 ml. Drug was administered in 0.2-ml volumes by gavage or in 0.1-ml volumes intraperitoneally twice daily at approximately 9:00 AM and 4:00 PM.

Viruses. The challenge viruses used in the animal experiments were mouse-adapted strains of influenza A/Beth/10/63 (H2N2) or influenza B/Maryland/1/59. In tissue culture studies, the myxoviruses were influenza A/PR/8/34 (H1N1), A/Beth/10/63 (H2N2), A/Hong Kong/1/68 (H3N2), A/Aichi/2/68 (H3N2), and B/Mass/3/66. The paramyxoviruses were the Sendai strain of parainfluenza type 1 and a parainfluenza type 2 of human origin. Among the rhinoviruses, types 2, 21, and 44 were selected for testing. Adenovirus types 3 and 7 and herpes hominis virus were selected as representative deoxyribonucleic acid-containing viruses.

RESULTS

Tissue culture. The results of a limited test of the antiviral spectrum of isoprinosine are given in Table 1. In these studies, the virus inoculum was added to the monolayer, followed immediately by the addition of drug in concentrations ranging from 10 to 100 μg/ml. Inhibition of the strains of myxoviruses tested occurred at concentrations ranging from 20 to 100 μg/ml.

The addition of 20 μg of isoprinosine/ml effected a decrease in the infectivity titer of influenza A/Beth/10/63 (H2N2) from $10^{5.5}$ to $10^{2.5}$ $TCID_{50}$, indicating 99.9% inhibition. The infectivity titer of influenza A/Hong Kong/8/68 (H3N2) was reduced from $10^{4.0}$ to $10^{1.4}$ $TCID_{50}$ when 50 μg of drug per ml was used, and to $10^{0.7}$ $TCID_{50}$ when 100 μg of isoprinosine/ml was added. Against influenza A/Aichi/1/68 (H3N2), the presence of as much as 100 μg of drug/ml had no effect when added to tissue culture simultaneously with as little as 1 $TCID_{50}$ of virus.

The addition of 100 μg of drug/ml to cell cultures simultaneously with $10^{5.8}$ $TCID_{50}$ of influenza B/Maryland/1/59 resulted in a 99.9% reduction in infectivity. Addition of isoprinosine in concentrations of 20, 40, 60, 80, and 100 μg/ml to cultures simultaneously infected with influenza B/Mass/3/66 resulted in a zone phenomenon of inhibition. The greatest reduction in infectivity (99%) occurred when 40 μg/ml was tested. Less reduction of virus infectivity was observed when 20, 60, or 80 μg/ml was tested, and almost no inhibition occurred when 100 μg/ml was used.

To determine whether isoprinosine obscured hemadsorption, which was used as the indicator of infection, rather than preventing viral replication, hemagglutination (HA) titers were measured in the supernatant fluid from cultures. Addition of drug in the HA test had no effect on the titer observed. When influenza B/Mass/3/66 was used, the reduction in the HA titer paralleled the zone phenomenon observed in infectivity; the greatest decrease was in the 50 μg/ml range (Fig. 1). When influenza A/Beth/10/63 (H2N2) was tested in like manner, the HA pattern paralleled the decrease in infectivity titer, but no zone phenomenon occurred.

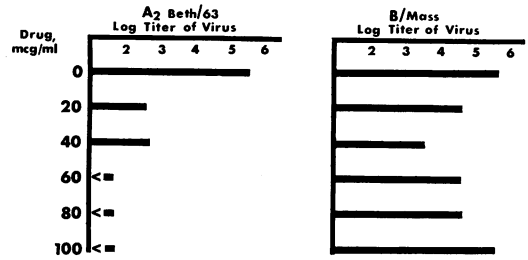

FIG. 1. *Isoprinosine inhibition of the infectivity of influenza viruses measured by hemagglutinin released from infected monkey kidney tissue culture.*

TABLE 1. *Antiviral spectrum of isoprinosine in infected tissue culture*

Virus strain	Tissue culture	Inhibitory concn (μg/ml)	Log decrease in infectivity of virus inoculum
Influenza			
A/PR/8/34	Primary rhesus monkey kidney	100[a]	—[b]
A/Beth/10/63	Primary rhesus monkey kidney	20	3.0
A/Hong Kong/8/68	Primary rhesus monkey kidney	50	2.6
A/Aichi/2/68	Primary rhesus monkey kidney	100	
B/Maryland/1/59	Primary rhesus monkey kidney	100	2.7
B/Mass/3/66	Primary rhesus monkey kidney	25–60 only[c]	2.0
Parainfluenza			
Type 1 (Sendai)	Primary rhesus monkey kidney	>100	
Type 2	Primary rhesus monkey kidney	>100	
Rhinovirus			
Type 2	WI-38	>100	
Type 21	WI-38	>100	
Type 44	WI-38	>100	
Adenovirus			
Type 3	Human embryonic kidney	>100	
Type 7	Human embryonic kidney	>100	
Herpes hominis	WI-38	75	2.0

[a] Lower concentrations not tested.
[b] Effect observed by HA titer drop of ≧ 1:64 to 1:16.
[c] Zone effect of inhibition. No inhibition at <25 or 100 μg/ml.

No antiviral effect was observed when 25, 50, 75, or 100 μg of isoprinosine/ml was tested against 0.3 to 3,160 $TCID_{50}$ of parainfluenza types 1 or 2. When 100 μg of drug/ml was added simultaneously with 0.2 to 6,310 $TCID_{50}$ of rhinovirus to cell cultures, no drug effect was seen at any virus dilution. No effect was observed in cells infected with similar amounts of adenovirus types 3 and 7.

Animal studies. The prophylactic effect of isoprinosine was tested in mice infected with influenza A. Oral doses of drug or placebo were begun 24 hr before virus challenge and given twice daily for 5 days. The virus inoculum was a mouse lung harvest containing 200, 20, or 2 LD_{50} of influenza A/Beth/10/63 (H2N2) given as nose drops to 20 animals per group. When an inoculum of 200 or 20 LD_{50} was used, there was no difference in mouse fatality between the drug-treated and placebo groups 21 days after challenge (Fig. 2), at which time the experiment was terminated. At no time during the test period was a delay in the time of death observed in the drug-treated group. When the challenge dose was 2 LD_{50}, survival of drug-treated animals was prolonged. From the 10th to the 13th day postchallenge, the difference was statistically significant ($P < 0.05$). However, there was no difference in the fatality rate of drug-treated or placebo-treated animals at the end of the test.

In therapeutic trials of isoprinosine, the drug was started 24 hr after infection and continued for 10 days. Each treatment group included 38 to 42 mice per virus dilution. When 40 LD_{50} of influenza A/Beth/10/63 (H2N2) was used as the inoculum, there was no difference in mouse fatality (Fig. 3) and no increase in the time of survival of drug-treated mice during the observation period. When 4 LD_{50} was used as challenge, a significant increase in the number of survivors was observed in the drug treatment group (38%) as compared to the control group (8%). This drug protection was evident from the first day mortality was observed and remained constant for 16 days until the termination of the experiment on day 21.

In another trial, drug was administered to mice infected with influenza beginning 24 hr after challenge and continued for only 4 days. No antiviral activity was detected when the virus inoculum was 40, 4, or 0.4 LD_{50}.

In the therapeutic assay of isoprinosine against influenza B/Maryland/1/59, the drug was given intraperitoneally twice daily in a dose of 1.2 g per kg per day. Treatment was started 24 hr after challenge and continued for 10 days. No antiviral effect was detected against a challenge of 20 to 40 LD_{50} (Fig. 4). When the challenge dose was 2 to 4 LD_{50}, the cumulative mortality by day 14 was reduced from 80 to 50%. Although the drug was not completely protective even against a low challenge dose of virus, the reduction of fatality was statistically significant. In two of three experiments in which the challenge dose of influenza B virus was less than 1 LD_{50}, the overall fatality rate among controls was 35%, and treatment with isoprinosine increased the fatality rate to 60%. This augmentation of lethality from a

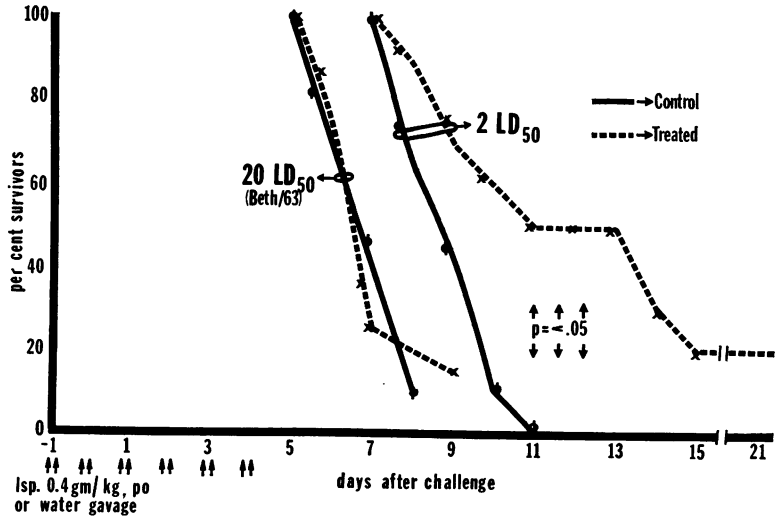

FIG. 2. *Oral isoprinosine prophylaxis of influenza A/Beth/10/63 (H2N2) in mice. The results are based on 40 mice per group, which is a composite of two separate experiments which were independently corroborative.*

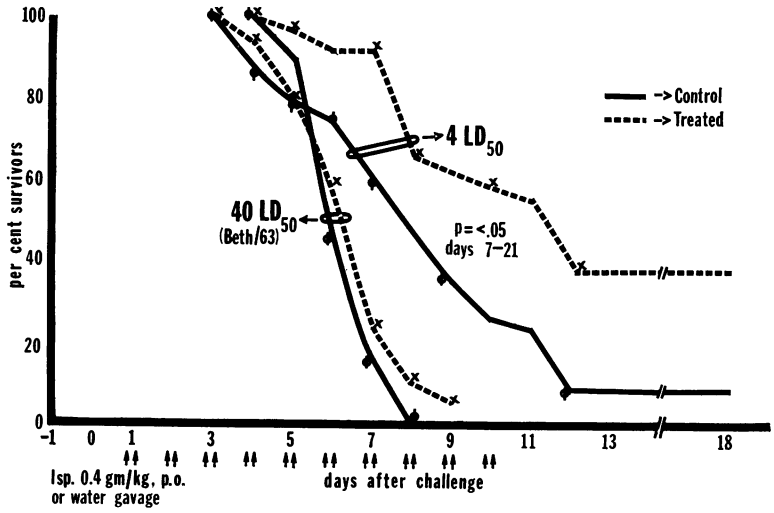

FIG. 3. *Oral isoprinosine treatment of influenza A/Beth/10/63 (H2N2) in mice. The results are based on 40 mice per group, which is a composite of two separate experiments which were independently corroborative.*

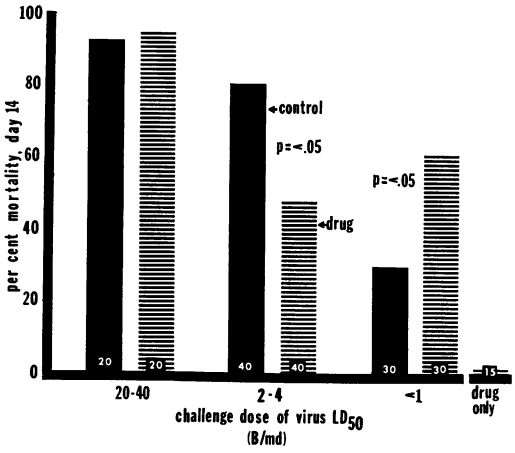

FIG. 4. *Results of treatment of influenza B virus infection in mice with isoprinosine in a dose of 1.2 g per kg per day given intraperitoneally for 10 days.*

small dose of influenza virus was statistically significant ($P < 0.05$). No deaths occurred in mice given only the drug.

DISCUSSION

Antiviral effects have been reported in isoprinosine-treated tissue cultures infected with influenza A virus, poliovirus type 3, and herpesvirus (E. R. Brown and P. Gordon, Proc. Fed. Amer. Soc. Exp. Biol. **29**:684, 1970; Brown and Gordon, Proc. Fed. Amer. Soc. Exp. Biol. **30**:242, 1971; Gordon, Brown, and Ronsen, Proc. Fed. Amer. Soc. Exp. Biol. **30**:242, 1971). In other observations, no reduction in the cytopathic effect (CPE) occurred in isoprinosine-treated tissue cultures infected with herpesvirus, vaccinia virus, poliovirus, or echovirus (T. W. Chang and L. Weinstein, Abstr. 11th Intersci. Conf. Antimicrob. Ag. Chemother., p. 29, 1971), but there was a 10- to 100-fold reduction in virus yield. Neither reduction in CPE nor decrease in virus yield was observed in cultures infected with mumps or measles virus.

In our preliminary survey, a difference in drug susceptibility was observed among the strains of influenza A tested. A 1,000-fold reduction in infectivity titer was observed when 20 μg of isoprinosine/ml was added to the most susceptible strain, influenza A/Beth/10/63 (H2N2). The more recent H3N2 strains of influenza virus were less susceptible. Although both the Hong Kong and Aichi strains were isolated in 1968, the infectivity titer of the former was reduced 300-fold by 50 μg of isoprinosine/ml, whereas no inhibition of the Aichi strain was observed at concentrations from 25 to 100 μg/ml. Many more strains must be tested to determine the frequency of the resistant isolates, their relation to antigenic variation, and the basis for the resistance. The results with hemagglutination and hemadsorption tests suggest that isoprinosine caused no inhibition in the release of virus, and the normal growth of drug-resistant strains tends to exclude direct inhibition of tissue cultures by isoprinosine as an explanation of the reduced titers of susceptible strains of influenza virus.

Others have reported antiviral effects in isoprinosine-treated mice challenged with influenza A/HK/8/68 (1). The drug was given intraperi-

toneally in a dose of 300 mg per kg per day starting 24 or 48 hr postchallenge and continuing until the termination of the experiment on day 16. Paradoxically, no effect was observed when drug was started on the day of virus challenge. Reduction in the amount of lung consolidation in influenza-infected mice also has been reported (Chang and Weinstein, Abstr. 11th Intersci. Conf. Antimicrob. Ag. Chemother., p. 29, 1971). The same authors reported preliminary results in hamsters infected with herpesvirus which indicated that isoprinosine caused a significant reduction of mortality and, in mice infected with vaccinia virus, a decrease in the number of tail lesions. Some workers have also observed that rhinovirus 1A (Newport Pharmaceuticals, *personal communication*) and adenovirus type 10 (Brown and Gordon, Proc. Fed. Amer. Soc. Exp. Biol. **29**:684, 1970) were susceptible to the action of isoprinosine. In our system, no antiviral effect could be demonstrated in drug-treated cell cultures infected with rhinovirus types 2, 21, and 44, or adenovirus types 3 or 7. These different results may reflect strain differences, inocluum size, or other factors, but they cast doubt on the likelihood of the utility of isoprinosine in the treatment of such infections.

With either prophylactic or therapeutic use of isoprinosine in mice, no antiviral activity against influenza A or B could be detected when the virus inoculum exceeded 20 LD_{50}. Thus, a high virus multiplicity obliterated any inhibitory activity of the drug. This effect should always be given consideration in the evaluation of drugs in investigational models of infection in animals. The infectious dose of naturally acquired respiratory infection is not known. Treatment begun 24 hr after infection was significantly protective when continued for 10 days but not when treatment was stopped after 5 days. Thus, the drug effect was not virucidal, and the duration of treatment was critical. A slight antiviral effect was recorded for mice treated for 5 days when treatment was begun 24 hr before challenge.

The definite therapeutic effect of the drug, when given by the oral route starting 24 hr after infection, is the most encouraging fact regarding the potential of isoprinosine as an anti-influenzal drug. Unfortunately, it is offset by the finding of resistant strains of virus, the limited range of virus dose against which any effective treatment is discernible, a possible zone effect related to drug dosage, and the observed potentiation of severity of mild influenza B infection. The augmented fatality rate of drug-treated mice challenged with less than 1 LD_{50} of influenza B/Maryland/1/59 indicates either a drug-stimulated increase in virus replication or some impairment of the host resistance by drug treatment. No death or overt illness occurred in animals given drug only. We have observed a similar increase in the fatality rate of mice infected with influenza when treated with other drugs having in vitro anti-influenzal activity. Potentiation of infection with small doses of infectious virus, therefore, may be a biological phenomenon rather than direct drug toxicity. The mechanism of the augmentation is unknown. The value of isoprinosine as an anti-influenzal drug in man, if any, would need to be resolved by well-controlled volunteer and field studies with the use of objective methods for evaluation and virus strains of known susceptibility to isoprinosine.

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