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Evaluation of Isoprinosine in Experimental Human Rhinovirus Infection

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The prophylactic efficacy of isoprinosine was evaluated in a double-blind fashion in volunteers challenged with two types of rhinovirus. In the rhinovirus 44 and 32 trials, each of 9 men received a placebo, and eight and 11 men received the drug, respectively. Oral isoprinosine, 6 g a day, was given for 2 days prior to intranasal challenge with 100 mean tissue culture infective doses of the virus and for 7 postchallenge days. In both trials the occurrence and severity of colds were greater in the placebo group, but the difference between the two groups was not significant. Higher antibody titers for both viruses and a greater number of rhinovirus 32 isolations were demonstrated in the drug group but without statistically significant differences. The prophylactic isoprinosine treatment may suppress the cold syndrome, but its effect was not convincingly apparent.

Isoprinosine (NPT 10381, *p*-acetamidobenzoic acid salt of inosine-dimethylaminoisopropanol in a 1:3 [vol/vol] mol ratio) has been reported as a broad-spectrum antiviral compound (E. R. Brown and P. Gordon, Fed. Proc. **30**:242), and it was active against infections caused by type A1 and A2 influenza virus strains, herpes zoster, and vaccinia (E. R. Brown and P. Gordon, Fed. Proc. **29**:684).

This evidence prompted the clinical evaluation of isoprinosine in experimentally induced rhinovirus infections of type 44 and 32 in man. Concurrent susceptibility investigations were conducted in tissue cultures with these viruses, as well as with other rhinovirus types. The results of the double-blind trials conducted in volunteers to assess the prophylactic efficacy of isoprinosine are reported here.

MATERIALS AND METHODS

Volunteers. Volunteers were healthy male prisoners (age range, 21 to 48). The serum-neutralizing antibody was titrated against 100 mean tissue culture infective doses ($TCID_{50}$) of challenge virus, and subjects who lacked serum antibody (titer, <1:2) or had a minimal titer (1:2) were enrolled. Each had the nature of the study explained to him, and informed consent was obtained. The protocols used in these studies were approved by the University of Maryland Committee on Clinical Investigations.

Challenge viruses. The second WI-38 cell passage rhinovirus 44 was used as the challenge virus. This challenge virus was previously used in a drug evaluation clinical trial (8). The fourth WI-38 cell passage of rhinovirus 32 was prepared in this laboratory from the stock culture (SF 693 strain 2nd WI-38 cell passage, kindly supplied by Vincent V. Hamparian of the Ohio State University, Columbus). The examination of these challenge pools revealed no contaminants.

Drug administration and viral challenge. Volunteers received orally either 1.5 g of isoprinosine at each meal and bedtime (6 g per day) or placebo tablets for 2 days prior to the intranasal inoculation of 100 $TCID_{50}$ of the challenge virus and for 7 consecutive postchallenge days. The drug and placebo tablets were supplied in coded bottles by Newport Pharmaceutical International, Inc., Newport Beach, Calif., and administered to volunteers in a double-blind fashion. A baseline observation period of 4 days was maintained to rule out incipient viral infection, and subsequently all subjects were kept in an isolation ward for 10 days after the challenge. Each subject was interviewed and examined by the physician once a day. Signs and symptoms of cold, i.e., rhinitis, rhinorrhea, nasal stuffiness, sore throat, pharyngitis, sneezing, cough, and others, were individually scored on a scale of 0 to 3+: 0, not present; 1+, mild; 2+, moderate; and 3+, severe. Each patient's chart was reviewed after discharge from the isolation ward, and the severity of induced rhinovirus illness of each subject was rated from 0 to 3+, according to the occurrence and severity of signs and symptoms and the duration of the illness. Hemogram, blood chemistries, including plasma and urine uric acid levels, and urinalysis were performed before, during, and after the isoprinosine treatment. The chest roentgenograms were done during the baseline period and before discharge from the ward.

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The evaluation of isoprinosine with rhinovirus 44 challenge was carried out in two separate trials in which seven and ten subjects were studied. A single trial with rhinovirus 32 challenge was performed with 20 subjects.

Virology materials and methods. The materials and procedures for the virus isolation from the nose and for the neutralizing antibody titrations of serum and nasal washing samples have been described elsewhere (8).

In vitro sensitivity of rhinovirus 44 and 32 (challenge viruses) and types 2, 24, 29, 30, 33, and 40 to isoprinosine was evaluated. The drug concentration, ranging from 1,000 to 1.96 μg/ml, was tested against 100 $TCID_{50}$ of each of the viruses. Also studied was reduction of the infectivity titer when rhinovirus culture was maintained in the medium containing 100 μg of the drug per ml. The details of this procedure have also been described (8). In both experiments, the cell cultures received fresh medium containing the drug once a day during a 5-day test period, and all WI-38 cell tube cultures were rolled at 35 C.

RESULTS

Rhinovirus 44 challenge. In this trial, nine and eight subjects received placebo and isoprinosine tablets, respectively.

(i) **Induced rhinovirus illness.** Five control subjects and one drug-treated subject developed colds after the rhinovirus 44 challenge (Table 1). One man in each group had a severe cold (3+). Four placebo controls had moderate illness (2+). The occurrence of the illnesses in the two groups, five of the nine placebo controls (56%) and one of the eight drug-treated men (13%), was not significantly different ($p > 0.10$, Fisher's exact test).

(ii) **Illness scores.** The mean illness scores for the two groups were tabulated daily from day −6 through day 10 (Fig. 1). Those who became ill developed typical rhinovirus illness, i.e., rhinitis, rhinorrhea, nasal stuffiness, sore throat, mild pharyngitis, sneezing, cough, and minimal cervical lymphadenopathy. The signs and symptoms increased in severity and frequency and reached the peak between days 2 and 3, with rapid resolution beginning day 4. The illness scores were higher in the placebo group throughout the observation period. However, the differences of the mean scores or of the individual sign or symptom scores on any postchallenge day were not significantly greater.

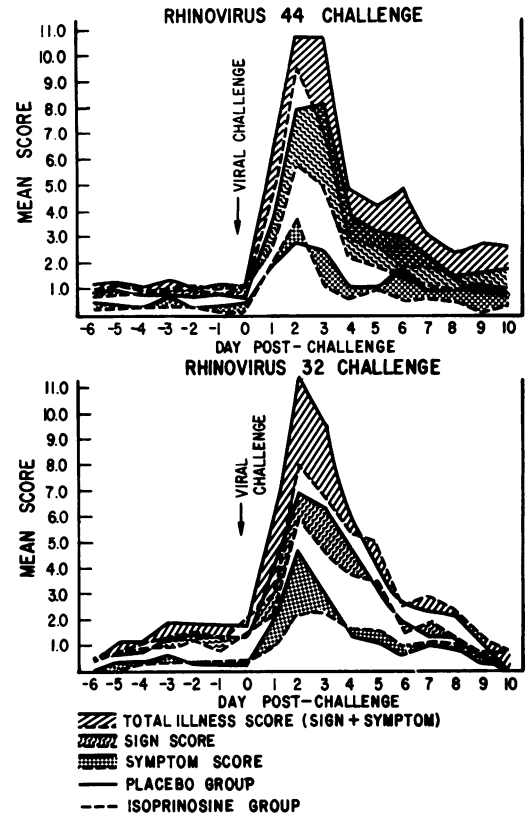

FIG. 1. *Differences in the total illness (sign plus symptom) scores, sign scores, and symptom scores between the isoprinosine-treated and placebo-treated groups.*

TABLE 1. *Occurrence and severity of rhinovirus illness in isoprinosine-treated and placebo-treated groups*

Degree of severity	Rhinovirus 44 challenge		Rhinovirus 32 challenge	
	Placebo	Isoprinosine	Placebo	Isoprinosine
Severe (3+)	1	1	1	1
Moderate (2+)	4	0	2	1
Mild (1+)	0	0	3	4
No illness (0)	4	7	3	5
Total	9	8	9	11

(iii) **Virus isolation.** All but two men in each group excreted the virus one or more times between days 1 and 10 (Fig. 2). No difference in the frequency of virus recovery between the two groups was noted on any day. The number of virus isolations from the two groups was similar, a total of 32 isolates in the placebo group with 3.6 isolates per individual and of 27 isolates in the drug group with 3.4 isolates per individual.

(iv) **Antibody responses.** Every subject in both groups except one placebo recipient showed significant antibody titer increases in their postchallenge blood (Fig. 3). The ranges of serum titers on day 30 for the placebo and drug

groups were <1:2 to 1:128 and 1:4 to 1:1024, respectively. The postchallenge geometric mean (GM) titers were higher in the drug-treated group. Days 15, 22, and 30 GM titers for the placebo and drug groups were 1:10, 1:15, and 1:13 and 1:29, 1:76, and 1:64, respectively, but the difference in mean titers between the two groups was not significant (p = 0.18725, 0.08111, and 0.07123, respectively, Student's t test).

About 80% of the subjects in both groups developed nasal secretory antibodies after viral challenge. The drug group had higher GM titers, but the difference was not significant (i.e., p = 0.12712 on day 30). The titer ranges on day 30 for the placebo and drug groups were <1:2 to 1:16 with the GM titer of 1:3.8 and <1:2 to 1:38 with the GM titer of 1:12.0, respectively.

Rhinovirus 32 challenge. In this trial, nine subjects received placebo tablets and 11 subjects received isoprinosine.

(i) **Induced rhinovirus illness.** Six subjects in each group developed colds after the viral challenge (Table 1). The typical rhinovirus illness was observed within 24 h postchallenge, which reached its peak on day 2, followed by rapid resolution beginning on day 3 (Fig. 1). Three placebo controls and two drug-treated men developed moderately severe colds (2+). One man in each group had a fever (38.6 C for the placebo subject and 37.9 C for the drug-treated man, both on day 2); hence, their scores were upgraded to 3+. Three men in the placebo group and four men in the drug group developed a mild illness (1+). Three controls and five drug-treated men were all asymptomatic. Although more placebo-treated men developed moderate (2+) and severe (3+) illnesses, the occurrence of these illnesses, three of the nine placebo men (33%) and two of the 11 drug-treated men (18%), was not significantly different (p > 0.10, Fisher's exact test). The occurrence of the illnesses (1+ to 3+) was similar in both groups: six placebo men (67%) and six drug-treated men (55%).

(ii) **Illness scores.** The illness scores were consistently higher in the placebo group during the first 3 days after the challenge (Fig. 1). However, the difference on any of these days was not statistically significant. The comparison of individual sign and symptom scores on postchallenge days between the two groups revealed no significant differences.

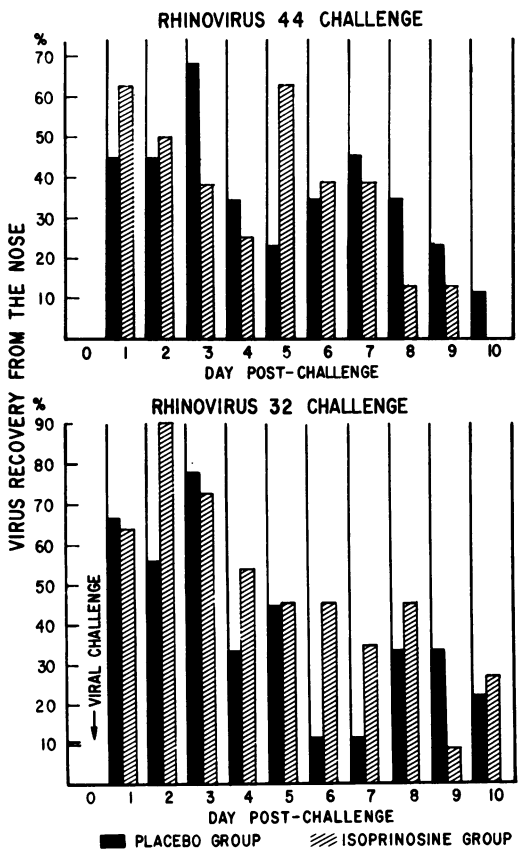

FIG. 2. *Frequency of virus shedding in the isoprinosine-treated and placebo-treated groups.*

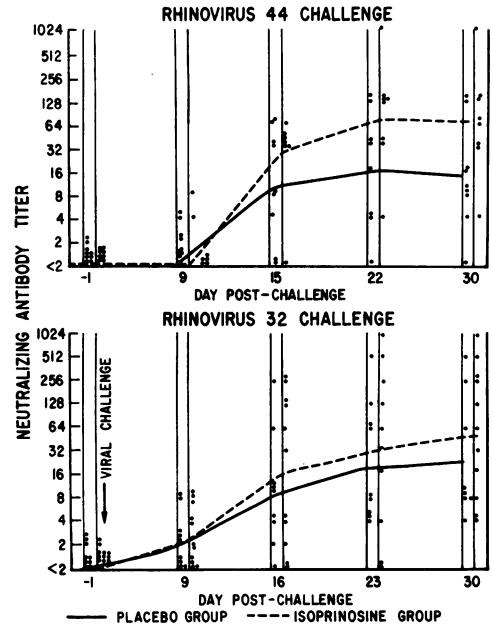

FIG. 3. *Serum antibody response pattern in the isoprinosine-treated and placebo-treated groups.*

(iii) **Virus isolation.** One or more virus isolations were made from all but one placebo control (Fig. 2). More drug-treated men shed the virus than the placebo-treated men on days 2, 4, 6, and 7, but the differences in the frequency were not significant on these days. The total numbers of virus isolations during the 10 postchallenge days for the placebo and drug groups were 35 (3.9 per individual) and 54 (4.9 per individual), respectively.

(iv) **Antibody responses.** All subjects in both groups showed significant postchallenge antibody titer increases (Fig. 3). The titer range for the placebo group was 1:4 to 1:512 with the GM titer for day 30 of 1:25, and that for the drug group was 1:4 to 1:1024 with the day 30 GM titer of 1:56. The postchallenge titers were higher in the drug group, but the difference was not significant (p = 0.36338, Student's t test).

Nasal secretory antibodies were demonstrated on day 30 in about 70% of the subjects in both groups. The titer range for the placebo groups was <1:2 to 1:11 with the GM titer of 1:3.3, and that for the drug group was <1:2 to 1:24 with the GM titer of 1:4.6.

Side effect of isoprinosine. Transient hyperuricemia and hyperuricosuria were evident in the drug-treated subjects during the treatment period (Table 2, 3), reflecting the increased production of uric acid, a breakdown product of isoprinosine. The plasma levels on the second and sixth treatment days and the urinary excretion on the second day were significantly higher in the drug group, but all plasma and urinary excretion values returned to normal within a few days after cessation of therapy. No arthritic symptoms were noted. No other side reactions attributable to isoprinosine treatment were found. All other laboratory results were within the normal limits.

In vitro activity of isoprinosine on rhinoviruses. The rhinovirus growth was not inhibited by isoprinosine at concentrations from 1,000 to 1.95 μg/ml in WI-38 cell cultures. There was no reduction in titers of rhinoviruses cultured in the presence of 100 μg of the drug per ml. No toxic effect of the drug on the cells was noted at concentrations up to 1,000 μg/ml.

DISCUSSION

Since the initiation of our clinical trial, additional reports on laboratory and clinical evaluation of isoprinosine have appeared. Muldoon, Mezny, and Jackson (6) reported that in tissue cultures *Herpes hominis* and type A influenza virus were inhibited at a drug concentration of 20 to 100 μg/ml. Among type A influenza strains, a variability in the inhibitory concentration of the drug was detected. The drug lacked an inhibitory action on parainfluenza 1

TABLE 2. *Plasma uric acid levels in the isoprinosine-treated and placebo-treated groups*

Test group	Plasma uric acid (mg, %)			
	Day −5	Day 2	Day 6	Day 11
	Mean SE[a]	Mean SE	Mean SE	Mean SE
Placebo	5.69 ± 0.29	5.47 ± 0.27	6.02 ± 0.25	5.69 ± 0.23
Isoprinosine	5.34 ± 0.21	7.56 ± 0.23	7.93 ± 0.21	6.02 ± 0.24
p (Student's t test)	0.32448	0.00002	0.00002	0.33323

[a] SE, standard error.

TABLE 3. *Urinary uric acid excretion in the isoprinosine-treated and placebo-treated groups*

Test group	Urine uric acid (mg/24 h)			
	Day −3	Day 2	Day 6	Day 11
	Mean SE[a]	Mean SE	Mean SE	Mean SE
Placebo	816.78 ± 100.25	656.67 ± 53.12	805.72 ± 112.46	592.00 ± 88.85
Isoprinosine	695.58 ± 55.54	1017.21 ± 57.51	1018.78 ± 99.80	436.61 ± 34.12
p (Student's t test)	0.28938	0.00007	0.16650	0.10673

[a] SE, standard error.

and 2, rhinovirus 2, 21, and 44, and adenovirus 3 and 7. Gordon and Brown (4) stated that in tissue cultures isoprinosine (10 μg/ml) was effective against type A influenza, herpesvirus (LU strain), poliovirus 3, and adenovirus 10, and the drug had a therapeutic effect against influenza and herpes infections in animals. They postulated that the antiviral effect of isoprinosine was the result of drug enhancement of host messenger ribonucleic acids to compete with viral messenger ribonucleic acids for host ribosomes, thus significantly reducing virus-directed protein synthesis. They believe that, because the drug acts on the host mechanism, its antiviral effect is broad-spectrum in nature. Chang and Weinstein (1) found a slight activity of isoprinosine in tissue cultures against *H. hominis* type 2, vaccinia, poliovirus 3, ECHO 11, and eastern equine encephalitis virus but no effect on measles, mumps, and western equine encephalitis virus. The drug reduced the morbidity and mortality in animals infected with herpes and type A influenza. Glasgow and Gallasso (3) reported the results of a coordinated isoprinosine study conducted in six laboratories in five animal species using 11 viruses. Contrary to the positive results of the above investigators, no therapeutic effect was demonstrated in this study in infections with 10 of these viruses. In tissue culture and trachea explants, the drug (10 μg/ml) did not affect the growth of influenza virus. The only favorable result was the suppression of fibroma virus lesions in rabbits. Because of the several contradictory in vitro and in vivo results, plus the above negative report with a variety of viruses, it is difficult to support the contention that isoprinosine is a potent, broad-spectrum antiviral agent.

Of the clinical trials thus far reported, E. A. Daiko (Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 11th, Atlantic City, p. 30, 1971) reported that the oral treatment of 4 g of isoprinosine a day for 12.5 days produced some beneficial results in patients with hepatitis-associated antigen-negative hepatitis while on treatment. This effect was not noted in hepatitis-associated antigen-positive patients. Longely, Dunning, and Waldman (5) reported that the oral treatment (2.5 g twice daily) given for 2 days prechallenge and for 8 days after the challenge with A (H3N2)/Hong Kong influenza virus did not protect volunteers from clinical influenza. Soto, Hall, and Reed (7) conducted a prophylactic trial in volunteers who were challenged with rhinovirus 9 and 31 simultaneously. Isoprinosine, 1.5 g four times a day, was given for 2 days before challenge and for 5 days postchallenge. No favorable evidence regarding the clinical picture, extent of virus shedding, or serological responses as a result of drug treatment was obtained. In tissue culture, the drug (125 μg/ml) in the medium showed no inhibition of either of the challenge viruses.

In our rhinovirus 44 clinical trial, a reduction in the occurrence and severity of induced rhinovirus illness was observed in the isoprinosine-treated group, although the difference in occurrence and severity between the drug and placebo groups was not significant. Because of difficulty in finding susceptible subjects for additional rhinovirus 44 trials, rhinovirus 32 was used. The tendency toward suppression of the cold syndrome was again observed in the trial with the rhinovirus 32 challenge. In these trials with rhinovirus 44 and 32, the illness rates for the placebo groups were almost the same, but those for the drug-treated groups were markedly different, i.e., 13% for rhinovirus 44 and 55% for rhinovirus 32. These differences seemed to have occurred by chance, because the number of study subjects in these two trials was small. A similar number (type 44) or a greater number (type 32) of virus shedders as detected in the drug-treated group. In both trials, the postchallenge antibody titers were higher in the drug group. This phenomenon was also reported in the trial with type A influenza (5). Combined results of the two trials were not different from those of each trial. The frequency of some of the symptoms was, however, amplified by combination and became significantly different between the placebo and drug-treated groups. Cough was noted more frequently on day 1 in the placebo group (eight men) than in the drug group (two men) ($p < 0.05$, Fisher's exact test). There were complaints of nasal stuffiness on day 4 by 12 drug-treated men and five placebo-treated men ($0.05 < p < 0.10$). Although the placebo group had higher scores, there was no difference in the frequency or severity of any sign or symptom at the height of the illness (days 2 and 3). The drug might have acted favorably at the onset of illness, but the effect was not substantial enough to suppress the signs and symptoms. In the present study, the challenge viruses, along with six other rhinovirus types, were not inhibited in vitro by isoprinosine, in accordance with the results of others (6, 7). In men with induced rhinovirus infections, the isoprinosine prophylactic treatment may have a weak suppressing effect on symptoms and signs, but this clinical effect does not seem to be based solely on its antiviral activity, because the virus growth was not affected. No analgesic or antipyretic properties have been attributed to this drug in animal

models, and there appeared to be no action on the vasomotor reactions to acetylcholine, histamine, and serotonine (5). The mechanism by which isoprinosine treatment may give rise to suppression of colds is unknown. Under different experimental circumstances, i.e., lower challenge dose and variation in drug dosing, perhaps a more favorable drug effect could be demonstrated. No adverse side effect was observed in the isoprinosine recipients in the present study or in other studies (5, 7). Fareed and Tyler (2) reported no clinical toxicity in 13 amyotrophic lateral sclerosis patients who received isoprinosine in doses of 3 to 6 g per day orally for a minimal of 3 months. Nevertheless, because of marked elevations of uric acid plasma and urine values during the course of drug administration, prolonged utilization of isoprinosine should be carried out with precaution.

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LITERATURE CITED

1. Chang, T. W., and L. Weinstein. 1973. Antiviral activity of isoprinosine *in vitro* and *in vivo*. Amer. J. Med. Sci. **265**:143-146.
2. Fareed, G. C., and H. R. Tyler. 1971. The use of isoprinosine in patients with amyotrophic lateral sclerosis. Neurology **21**:937-940.
3. Glasgow, L. A., and G. J. Galasso (ed.). 1972. Isoprinosine: lack of antiviral activity in experimental model infections. J. Infect. Dis. **126**:162-169.
4. Gordon, P., and E. R. Brown. 1972. The antiviral activity of isoprinosine. Can. J. Microbiol. **18**:1463-1470.
5. Longley, S., R. L. Dunning, and R. H. Waldman. 1973. Effect of isoprinosine against challenge with A(H3N2)/Hong Kong influenza virus in volunteers. Antimicrob. Ag. Chemother. **3**:506-509.
6. Muldoon, R. L., L. Mezny, and G. G. Jackson. 1972. Effects of isoprinosine against influenza and some other viruses causing respiratory diseases. Antimicrob. Ag. Chemother. **2**:224-228.
7. Soto, A. J., T. S. Hall, and S. E. Reed. 1973. Trial of the antiviral action of isoprinosine against rhinovirus infection of volunteers. Antimicrob. Ag. Chemother. **3**:332-334.
8. Togo, Y., A. R. Schwartz, and R. B. Hornick. 1973. Failure of a 3-substituted triazinoindole in the prevention of experimental human rhinovirus infection. Chemotherapy **18**:17-26.