Antiviral Effect of 3,4-Dihydro-1-Isoquinolineacetamide Hydrochloride in Experimental Human Rhinovirus Infection

YASUSHI TOGO, ANDREW R. SCHWARTZ,¹ AND RICHARD B. HORNICK

*Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine,
Baltimore, Maryland 21201*

Received for publication 27 July 1973

Double-blind trials were conducted in volunteers to evaluate the efficacy of the prophylactic 3,4-dihydro-1-isoquinolineacetamide hydrochloride (DIQA) treatment against rhinovirus type 24 challenge. Ten men received a 7-day course of DIQA treatment and 11 men received a placebo. The intranasal viral challenge dose was 10 mean tissue culture infective doses. The oral administration of 1 g prechallenge and 2 g a day for 6 consecutive postchallenge days did not prevent the development of colds. Nine drug-treated men and 10 controls developed rhinovirus illness. However, the illnesses of the drug-treated men were mild. Rhinorrhea occurred less frequently and was more mild in the drug-treated group. The challenge virus was recovered from 80% of these subjects in both groups, but almost twice the number of challenge viruses were isolated from the controls than from the drug-treated men. The prophylactic DIQA therapy appears to suppress the cold syndrome and to reduce virus excretion, although its effect is marginal. Additional clinical trials are warranted to confirm the antirhinoviral effect of this drug.

An antiviral compound, 3,4-dihydro-1-isoquinolineacetamide hydrochloride (DIQA; Fig. 1) has been shown to be prophylactically effective in mice against infections produced by influenza (A/PR/8/34 and A/Japan/305/57), ECHO 9 (Coxsackie A23), herpes simplex, encephalomyocarditis (Columbia SK), St. Louis encephalitis, and West Nile viruses. The drug exhibited prophylactic and therapeutic activity against mumps infection in monkeys and effectively suppressed the development of herpes keratitis in rabbits, but the drug did not exert any effect on these viruses in tissue cultures. DIQA was active against Coxsackievirus B1 and vaccinia in tissue cultures, but was ineffective in vivo (1-3, 6).

Two isoquinoline derivatives (UK 2054 and UK 2371) inhibited the in vitro growth of viruses belonging to the myxovirus and picornavirus groups (4). In experimentally induced

types A and B influenza in man, the prophylactic treatment by UK 2371 (7) and by UK 2054 (5) significantly reduced the occurrence of the illness and suppressed the signs and symptoms. UK 2054, which showed an in vitro inhibitory action on rhinovirus type 9, failed to prevent experimental human infection of this virus (8).

Although tissue culture and animal study results to support an antirhinoviral effect of DIQA were not available, a double-blind prophylactic trial was conducted to assess the activity of this compound against a rhinovirus in man. This paper presents the results of this trial and those of concurrent in vitro evaluation of the activity of DIQA on rhinoviruses.

MATERIALS AND METHODS

Volunteers. Twenty-one healthy, male prisoner volunteers who lacked serum antibody or had a minimal antibody titer against challenge virus were enrolled. The nature of the study was explained to the volunteers, and informed consent was obtained. The range in ages was between 21 and 42 years.

Challenge virus. The fourth passage in WI-38 cell

¹ Present address: Division of Infectious Diseases, Department of Medicine, Hahnemann Medical College & Hospital of Philadelphia, Philadelphia, Pa. 19102.

FIG. 1. Chemical structure of DIQA (3,4-dihydro-1-isoquinolineacetamide hydrochloride).

culture of rhinovirus type 24 was used. The stock culture (SF 680 strain, second WI-38 passage) was kindly supplied by Vincent V. Hamparian of the Ohio State University. The passage and preparation of the challenge pool were carried out in this laboratory. The examination of the challenge pool revealed no contaminants.

Drug administration and viral challenge. DIQA and placebo capsules were supplied by Hoffmann-La Roche, Inc. in coded bottles and were administered to study subjects in a double-blind fashion. On the day before the challenge, each man received one capsule (500 mg) 0.5 h before the evening meal and at bedtime. On the 2nd day of drug treatment, about 2 h before the morning medication, the challenge virus was inoculated. A total of 1.0 ml containing approximately 10 mean tissue culture infective dose of the virus was instilled by medicine dropper into the nostrils (0.5 ml per nostril). On this and the subsequent 5 days, each subject received one capsule 0.5 h before each meal and at bedtime (2 g a day).

Drug evaluation trial. Volunteers were admitted to the isolation ward 5 days prior to the viral challenge. Oral temperature and pulse and respiration rates were recorded every 4 h, except at 4 A.M. Each study subject was examined by a physician once a day throughout a 19-day study period. Signs and symptoms were individually scored on a scale of 0 to 3+: 0, not present; 1+, mild; 2+, moderate; and 3+, severe. After discharge from the ward, each subject's chart was reviewed, and the severity of induced rhinovirus illness was rated from 0 to 3+ according to the occurrence and severity of signs and symptoms and the duration of illness. Urinalysis and a complete blood cell count, including hemoglobin and erythrocyte sedimentation rate, were performed before, during, and after the DIQA treatment. Urea nitrogen, glucose, serum glutamic-oxalacetic transaminase, creatinine, calcium, phosphorus, sodium, potassium, chloride, and carbon dioxide blood chemistries were also done. Electrocardiogram and chest roentgenogram were done during the base line period and before the discharge from the ward.

Two clinical trials were conducted: the first trial was performed in June with 16 men, and the second trial was in February with five men.

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 (9).

In vitro sensitivity of rhinovirus types 24, 1B, 32,

and 44 to DIQA was evaluated. The drug concentrations, ranging from 1,000 to 1.95 $\mu\text{g/ml}$, were tested against 100 TCID₅₀ of each of the viruses. The reduction of the infectivity titer of the rhinovirus which was cultured with the medium containing 100 $\mu\text{g/ml}$ of DIQA was also studied. The details of this procedure have also been described (9). In order to determine the direct activity of DIQA on the rhinoviruses, the drug solution and each of the viruses were mixed together to make the drug concentration in the mixture 1,000 $\mu\text{g/ml}$. The mixtures were left at 37 C for 1 h, after which the viruses were titrated in WI-38 cell tube cultures, and the titers were compared to those of controls. WI-38 cell tube cultures were rolled at 35 C during these experiments.

RESULTS

Of the 21 subjects, 10 received DIQA and 11 received a placebo. Age and body weight distributions were comparable in the two groups.

Induced rhinovirus infection. Nine of the 10 DIQA-treated men and 10 of the 11 placebo-treated men developed mild to moderately severe colds after the intranasal rhinovirus 24 challenge (Table 1). One man in each group had a 1-day elevation of body temperature (38.3 C [101 F] for the drug-treated man and 37.7 C [99.8 F] for the placebo-treated man), hence their scores were upgraded to 3+.

In the DIQA-treated group, six men developed 1+ to 2+ illness accompanied by seroconversion with one or two type 24 virus isolations between days 1 and 4. In the two men who had a mild cold, no challenge virus was isolated, but an antibody titer rise was demonstrated. One man who developed 2+ illness with fever excreted type 24 virus on days 1 and 2. However, his convalescent titers for the type 24 virus and type 1B virus (concurrent rhinovirus detected in the first study) remained unchanged at <1:2. One subject (no. 8, H.G.) was asymptomatic throughout the study period, but excreted type 1B virus on day 1 and challenge virus on days 2, 5, and 9. He subsequently developed antibodies for both viruses. Type 1B virus was carried by a placebo subject in the first study, which appears to have been transmitted to this drug-treated man.

In the placebo group, seven men developed mild to moderately severe illnesses with type 24 virus excretion and significant type 24 serum titer increases. Three men who were either asymptomatic or mildly ill excreted the challenge virus on day 3. However, their postchallenge serum titers for type 24 virus remained undetectable. One man (no. 18, C.W.), who was in the first study and developed a mild illness, did not excrete the challenge virus, but showed a significant titer rise for this virus. Type 1B

TABLE 1. *The severity of induced rhinovirus type 24 illness, virus recovery, and serum antibody titers in the DIQA-treated and placebo-treated subjects*

Subject	Severity of illness	Virus isolation ^a	Antibody titer ^b	
			24	1B
DIQA-treated group				
Study no. 1				
1. J.M.	2+	24 (4)	<2/256	<2/ <2
2. J.H.	1+	24 (1)	2/256	<2/ <2
3. J.R.	1+	24 (2)	<2/64	8/16
4. R.W.	1+	24 (2)	<2/32	8/4
5. E.M.	1+	0	<2/16	16/16
6. A.Z.	1+	0	<2/32	2/2
7. A.R.	3+	24 (1, 2)	<2/ <2	<2/ <2
8. H.G.	0	1B (1), 24 (2, 5, 9)	<2/8	<2/64
Study no. 2				
9. G.M.	2+	24 (4)	2/1,024	
10. M.C.	1+	24 (2, 3)	<2/8	
Placebo-treated group				
Study no. 1				
11. C.A.	2+	24 (2)	<2/16	8/8
12. J. Bo.	2+	24 (2)	<2/128	8/8
13. R.M.	2+	24 (1, 2)	<2/8	<2/ <2
14. L.O.	1+	24 (3, 6)	<2/512	8/8
15. J. Ba.	1+	24 (3)	<2/ <2	4/4
16. J.T.	1+	24 (3)	<2/ <2	32/64
17. C.S.	0	24 (3)	<2/ <2	4/4
18. C.W.	1+	1B (0, 1, 2, 3)	<2/32	4/1,024
Study no. 2				
19. E.N.	3+	24 (1, 2, 9)	<2/16	
20. D.P.	2+	24 (1, 2, 4)	<2/16	
21. K.D.	1+	24 (2, 3, 4, 5, 6, 7, 8)	<2/1,024	

^a 24, Type 24; 1B, type 1B; 0, no virus isolated. The number in parentheses is the postchallenge day on which the virus was isolated.

^b Prechallenge titer per 4-week postchallenge titer. The titers are expressed as the reciprocal of serum dilution.

virus was isolated from his nasal washing samples on days 0, 1, 2, and 3, and a serum titer increase for this virus was also demonstrated. No virus had been recovered on the 3rd day prior to challenge. The preexisting type 1B infection did not interfere with the development of type 24 infection in this man. This type 1B virus was transmitted to only one drug-treated subject among the six sero-negative contacts.

Illness scores. There was no difference between the two groups in the frequency of the illnesses. The frequency of moderately severe illnesses (2+ and 3+) was also not different. However, the total illness (sign plus symptom) scores and total symptom scores were consistently higher in the placebo group on days 2, 3, 4, and 5, although these differences were not statistically significant (Fig. 2). Some signs and symptoms, i.e., rhinorrhea, nasal stuffiness, headache, cough, and sneezing occurred more frequently in the placebo controls. Rhinorrhea was observed in eight controls and three drug-treated men on day 3 and in six controls and two drug-treated men on day 4. The placebo sub-

FIG. 2. *The differences in the total illness (sign plus symptom) scores, sign scores, and symptom scored between the DIQA-treated and placebo-treated groups.*

jects had a more severe rhinorrhea on day 3: the mean score for rhinorrhea was higher in the placebo group on this day ($P < 0.05$, Student's *t*-test).

Virus isolation. The challenge virus was isolated from eight drug-treated men (80%) and nine placebo controls (82%) (Table 1). The total numbers of virus isolates were 21 type 24 viruses and 4 type 1B viruses for the placebo group and 12 type 24 viruses and 1 type 1B virus for the drug group. Therefore, almost twice the number of type 24 viruses were isolated from the placebo group than from the drug group. On day 3, five placebo-treated men and one drug-treated man excreted type 24 virus. The frequency of the virus isolation from men in both groups on any day during 10 days after the challenge, including day 3, was not significantly different.

Serum and nasal secretory antibody titers. All but one man in the drug-treated group (90%) and three men in the placebo group (73%) showed serum neutralizing antibody titer increases for the challenge virus (Table 1). The 4-week postchallenge titers were in a range of $<1:2$ to $1:1,024$ for both groups. The geometric mean titers for the drug and placebo groups were $1:39$ and $1:19$, respectively, which were not significantly different. Eight drug-treated men (80%) and six placebo controls (55%) developed nasal secretory antibody. The titer ranges were from $<1:2$ to $1:21$ with a geometric mean titer of $1:4.8$ for the drug-treated group and from $<1:2$ to $1:23$ with a geometric mean titer of $1:2.7$ for the placebo group.

Concurrent rhinovirus infection. Concurrent rhinovirus type 1B infection was found in the first study. Type 1B virus was isolated from one each of the controls and the drug-treated subjects, both men having serum titer increases for this virus. Type 1B virus was not isolated from the remaining men. Of these, four drug-treated men and one placebo control remained antibody-free and the rest had unchanged serum titers at the 4th week postchallenge (Table 1).

Side effect of DIQA. No adverse reaction attributable to the ingestion of 2 g a day of DIQA for 7 days was noted. One placebo-treated man developed transient urticarial lesions on his hands and forearms on day 5. The discoloration of urine (orange to red amber) was noted in five men while taking DIQA and in one man who received a placebo. All hematology, blood chemistry, and urinalysis results were within the normal limits.

In vitro activity of DIQA on rhinoviruses. DIQA did not inhibit the growth of all four types of rhinovirus at concentrations up to $1,000 \mu\text{g/ml}$. Cytotoxic effect was noted at the drug concentration of $200 \mu\text{g/ml}$ and greater. No reduction in virus titers was observed in the cultures maintained with DIQA. Direct inac-

tivating action of DIQA against rhinoviruses was not detected in the contact experiment.

DISCUSSION

The inoculation of 10 TCID_{50} of rhinovirus type 24 resulted in the development of colds of various severity in 90% of placebo-treated men. Although the challenge dose was small, the incubation period was not extended, and the peak of the illness was on the 3rd postchallenge day. However, the frequency of the virus recovery from each man was low, i.e., nearly one-half of the subjects excreted the virus only once during a 9-day postchallenge period. The antibody response was undetectable in 3 of the 11 placebo controls. These three men excreted the virus one time, and two of them had a mild illness. The viral challenge dose should be minimal to allow the test drug a fair chance to exert its action against the virus. If the dose is exceedingly small, no illness will be produced and should the illness be induced, enough data in the clinical or laboratory parameters, or both, to insure that the illness was caused by the challenge virus will not be attainable. Thus, 10 TCID_{50} of type 24 virus appears to be the appropriate dose for the investigation of the drug antiviral effect with the number of subjects employed in this study.

DIQA did not inhibit the growth of four types of rhinovirus, including the challenge virus under the conditions of the experiments employed in the present study. The inhibitory activity of an isoquinoline derivative (UK 2054) against rhinovirus was demonstrated in HeLa cell cultures, but not in HEL-218 cell cultures (a semicontinuous line of human embryonic lung fibroblasts similar to WI-38 cells) (7). Therefore, further studies using HeLa cell cultures or a more sensitive technique, such as the plaque-reduction technique, are warranted. Other isoquinoline derivatives (UK 2371 and UK 2054) were shown to have no direct inactivating effect on viral infectivity, but were capable of inhibiting cytopathic effects and multiplication of rhinoviruses. These compounds had different modes of action on myxoviruses, i.e., direct inactivation of viral infectivity and the inability to interfere with the reproductive cycle (4). DIQA had no interferon-inducing action in mice, and its effect on Columbia SK virus appeared to be on a step in the virus-cell interaction between adsorption and replication (6).

Concurrent rhinovirus type 1B asymptomatic infection was noted in the first trial. The type 1B virus carried by the placebo-treated men was transmitted to only one drug-treated man

among the five men in the drug-treated group and the one man in the placebo-treated group who did not have circulating antibody to type 1B virus. DIQA might have prevented the spread of type 1B virus, but because of the small number of subjects, no conclusion can be drawn.

Although the DIQA treatment did not prevent the development of colds, the illness was more mild in the drug-treated group and, in particular, rhinorrhea occurred less frequently and was more mild in this group. The total number of virus isolates was smaller in the drug-treated group. The prophylactic DIQA treatment appears to suppress the cold syndrome and to reduce virus excretion in man, although its effect is marginal. Additional clinical trials are warranted to confirm the anti-rhinoviral effect of this drug.

ACKNOWLEDGMENTS

The present study was supported by grants from Hoffmann-La Roche, Inc., Nutley, N.J. We thank Suketami Tominaga of the Department of Preventive Medicine, University of Maryland School of Medicine for the statistical analysis of the results.

LITERATURE CITED

1. Grunberg, E., and H. N. Prince. 1968. The antiviral activity of 3,4-dihydro-1-isoquinolineacetamide hydrochloride *in vitro*, *in ovo* and in small laboratory animals. *Proc. Soc. Exp. Biol. Med.* **129**:422-430.
2. Grunberg, E., and H. N. Prince. 1970. Experimental methodology and the search for effective antiviral agents. *Ann. N.Y. Acad. Sci.* **178**:122-130.
3. Hoffman-La Roche, Inc. Clinical data sheet, Ro 5-3369, antiviral agent. Hoffmann-La Roche, Inc., Nutley, N.J.
4. Larin, N. M., A. S. Beare, M. P. Copping, C. R. McDonald, J. K. McDougall, B. Roberts, and J. B. Smith. 1968. Spectrum and characteristics of the virus-inhibitory action of two isoquinoline derivatives. *Antimicrob. Ag. Chemother.* 1967, p. 646-653.
5. Meenan, P. N., and I. B. Hillary. 1969. Prophylaxis of influenza B with UK 2054. *Lancet* **2**:614-615.
6. Murphy, B. R., and L. A. Glasgow. 1970. The antiviral activity of 3,4-dihydro-1-isoquinolineacetamide hydrochloride on Columbia SK virus infection in mice and tissue culture. *Ann. N.Y. Acad. Sci.* **178**:255-273.
7. Reed, S. E., A. S. Beare, M. L. Bynoe, and D. A. J. Tyrrell. 1970. Studies of isoquinoline derivatives (U.K. 2371 and U.K. 2054) in respiratory infections of volunteers using influenza viruses, a parainfluenza virus and a rhinovirus. *Ann. N.Y. Acad. Sci.* **178**:760-769.
8. Reed, S. E., and M. L. Bynoe. 1970. The antiviral activity of isoquinoline drugs for rhinoviruses *in vitro* and *in vivo*. *J. Med. Microbiol.* **3**:346-352.
9. 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.