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Effect of Isoprinosine Against Challenge with A(H_3N_2)/Hong Kong Influenza Virus in Volunteers

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Volunteers were challenged with A(H_3N_2)/Hong Kong/8/68 influenza virus while being given prophylaxis with either isoprinosine or placebo in a double-blind experiment. Isoprinosine, which had demonstrable antiviral activity in animal models, did not appear to protect humans from clinical influenza. The only beneficial effect of the drug observed was a slight, but significant, reduction in virus shedding.

Isoprinosine, the paracetamido-benzoic acid salt of inosine dimethylaminoisopropanol (1:3 molar ratio), has shown antiviral activity in tissue culture and in animals (2, 3; E. R. Brown and P. Gordon, Fed. Proc. **29**:684, 1970; E. R. Brown and P. Gordon, Bacteriol. Proc., p. 225, 1971). Tissue culture studies have revealed antiviral activities against poliovirus (type 3), herpesvirus (LU strain), adenovirus type 10, and influenza A_2 and A/PR_8 virus (3; Brown and Gordon, Bacteriol. Proc., p. 225, 1971). Antiviral activity was not shown in animal models challenged with representatives of herpesvirus, picornavirus, rhabdovirus, myxovirus, poxvirus, and coronavirus groups (2).

Antiviral activity has been shown in mice given oral isoprinosine against several influenza strains (4; Brown and Gordon, Fed. Proc. **29**:684, 1970); however, the results differed dependent upon the strain (4). The effectiveness of the anti-influenza activity in mice has been questioned in a recently reported study (2).

In a preliminary investigation conducted by Newport Pharmaceuticals (Newport Beach, Calif.), 20 adult males were given 1 to 6 g/day over a 4- to 6-week period. No unusual subjective complaints or alterations in physical exam, electrocardiogram, electroencephalogram, or hemogram were observed. Mildly elevated serum uric acids were the only abnormal biochemical determinations noted; there has been no evidence of clinical gout.

To investigate the antiviral efficacy of the compound, we challenged volunteers with A(H_3N_2)/Hong Kong influenza virus 48 h after initiation of either isoprinosine prophylaxis or placebo in a double-blind experiment.

MATERIALS AND METHODS

Conduct of study. Thirty adult volunteers of either sex were selected from the community on the basis of low or absent neutralizing antibody. Serum specimens were tested against 32 to 320 $TCID_{50}$ of A(H_3N_2)/Hong Kong/8/68 influenza virus (1); volunteers were excluded from the study if hemadsorption-inhibition-neutralizing activity was present in a 1:8 dilution of serum.

After a medical history, physical examination, complete blood count, urinalysis and serum uric acid, and an informed consent, volunteers were given either isoprinosine (2.5 g orally twice daily) or placebo tablets in a double-blind manner, the tablets having been coded by the drug manufacturer. Patients were isolated in the Clinical Research Center at the J. Hillis Miller Health Center of the University of Florida College of Medicine 48 h after treatment had been started. The volunteers were then challenged with intranasal instillation of 10^5 $TCID_{50}$ of A(H_3N_2)/Hong Kong influenza virus. The virus was obtained from Y. Togo, University of Maryland Medical School. In previous studies, this virus has been shown to infect about 50% of the negative individuals at this dose. Isoprinosine treatment, 2.5 g twice daily, was given for a total of 10 days.

Volunteers were examined twice daily, by two of the investigators independently, and a scale of 0 to 4 was used to record the severity of 12 symptoms and 7 signs consistent with influenza illness. Temperature, pulse, respiratory rate, and blood pressure were obtained four times daily by members of the nursing staff of the Clinical Research Center.

Laboratory methods. Virus isolation from throat gargles was attempted just prior to challenge and twice daily for the ensuing 3 days. Specimens for virus

isolation were inoculated on rhesus monkey kidney tissue culture in duplicate. After 72 h, the tissue culture supernatant fluid was passed onto fresh rhesus monkey cells. Presence of virus was indicated therefore by hemadsorption in the four tissue culture tubes per gargle. Each person underwent eight throat gargles during the study, representing a potential total of 32 isolates per person.

Hemadsorption-inhibition-neutralizing activity, as previously described (1), was again determined on the fifth and twenty-first days after viral challenge.

On the seventh day of drug therapy, hemogram and uric acid were again obtained. In addition, 10 of the 30 subjects were tested for 24-h uric acid clearances.

RESULTS

Fifteen patients received isoprinosine, and 15 received placebo. The groups were similar with regard to race, sex distribution, age, weight, and serological status prior to virus challenge (Table 1).

Clinical response. Seven objective parameters (nasal mucosal erythema and mucus, posterior, pharyngeal, and tonsillar pillar erythema, tympanic membrane erythema, palpable lymphadenopathy, and auscultatory findings pertinent to the lungs) and 12 complaints (sore throat, stuffy nose, runny nose, ear problem, cough, chill, headache, swollen glands, muscle ache, sore joints, nausea, and diarrhea) were evaluated and scored 0 to 4. Scores are presented in Fig. 1 and represent the total score for drug and placebo groups at regular time intervals after virus challenge. There was virtually no difference in the total scores of the two groups ($P > 0.1$).

Temperature elevations were considered separately and are presented in Fig. 2. The total degrees Celsius above 37 C for the individuals in each group were totaled at each time period (8 a.m., 12 noon, 4 and 8 p.m.). Influenza virus challenge was carried out at noon on day 1. Two patients in the placebo group received aspirin for discomfort, which may have lessened the total temperature rises at 64, 84, 88, and 92 h. There was no statistical difference between the temperature elevations in the two groups (P = 0.1, Student's t test).

TABLE 1. *Age, sex, and antibody response of volunteers*

Challenge group	Male/ female	Avg age (years)	Geometric mean rise in serum neutralizing antibody titer
Placebo	11/4	24.0	14-fold
Isoprinosine	10/5	20.6	24-fold

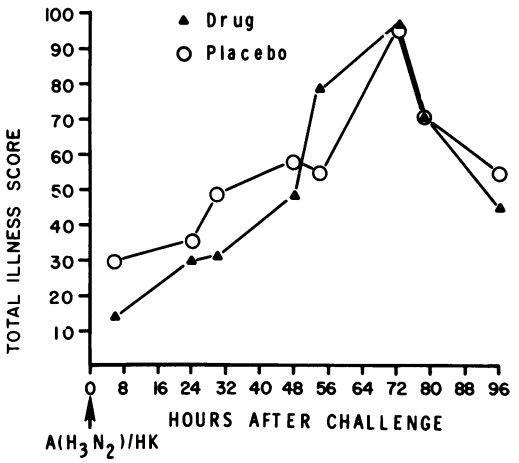

FIG. 1. *Total clinical illness scores (see text) of volunteers challenged with A(H_3N_2)/Hong Kong influenza virus.*

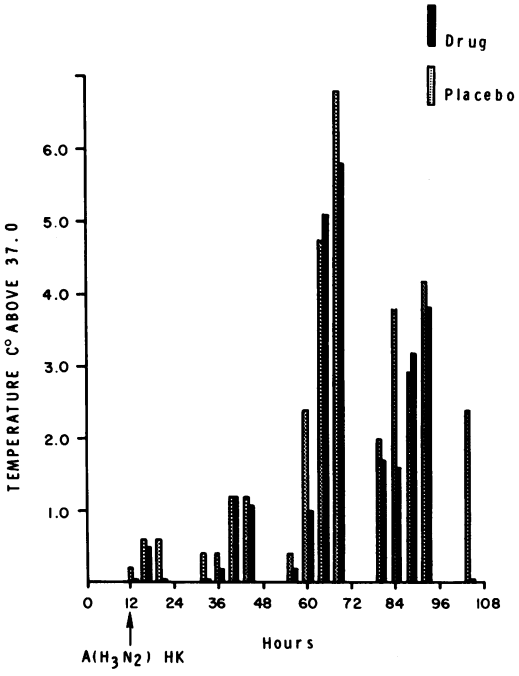

FIG. 2. *Total temperature elevation (above 37 C) in volunteers at various time intervals after influenza virus challenge (at 12 noon on day 1).*

Overall, on the basis of the criteria of two or more respiratory signs and/or symptoms and fever (temperature >37.5 orally), five volunteers in the isoprinosine group and seven in the placebo group were declared ill.

Laboratory results. Influenza A(H_3N_2)/HK isolates are tabulated in Fig. 3. Onset of virus

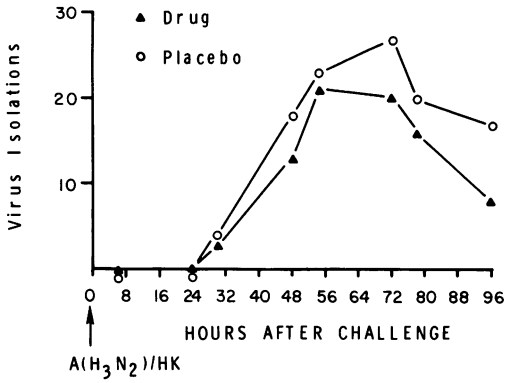

FIG. 3. *Virus shedding in volunteers challenged with A(H_3N_2)/Hong Kong influenza virus (0 = virus challenge). Total differences significant by chi square analysis ($P = 0.05$).*

shedding, peak time of shedding, and duration of shedding were similar in both groups; however, the total number of virus isolates for the isoprinosine group was significantly less than for the control by the chi square analysis ($P = 0.05$). Ten volunteers, seven in the isoprinosine group and three in the control group, had no virus isolated on any occasion ($P > 0.05$).

Serological conversion was demonstrated by 14- and 24-fold rises in geometric mean titers of serum-neutralizing antibody, respectively, in placebo and isoprinosine groups ($P > 0.1$). Seven volunteers, two in the isoprinosine group and five in the control group, did not show a significant rise in serum antibody ($P > 0.05$).

Side effects. Initial and post-treatment complete blood count (hematocrit, leukocyte count, differential), urinalysis, and serum uric acids were compared. The mean serum uric acid level in the isoprinosine groups prior to beginning the drug was 5.7. After 6 days of therapy, the mean serum uric acid level had risen to 7.9. No elevations of serum uric acid were noted in controls. In the isoprinosine group, no clinical gout was present, no abnormalities in renal function were observed, and serum uric acids had returned to normal limits for all subjects within 10 days of discontinuation of therapy. No abnormalities were observed in the hemogram, and all subjects were without subjective or additional objective findings attributable to isoprinosine therapy.

DISCUSSION

Others have demonstrated anti-influenza effects in mice against influenza A (PR_8 strain) and A_2 (Bethesda strain) with both inosine and complexes of inosine with alkylamino alcohols (as diethyl or dimethylamino-isopropanol). The compounds were as effective orally as by intraperitoneal injection, and 50% protection was achieved with treatment initiated as late as 7 days after intranasal inoculation, whereas 80% mortality was observed in controls (Brown and Gordon, Fed. Proc. **29**:684, 1970). In mice given influenza A_2 intranasally, isoprinosine administered intraperitoneally beginning on days 0, 1, and 2 showed therapeutic action; the effective dose was 500 mg/kg given twice daily (3). Prolonged mouse survival has been demonstrated with a low (2 LD_{50}) dose challenge with A(H_2N_2)/Bethesda/10/63, but not with 20 or 200 LD_{50}, in a trial in which isoprinosine was given prophylactically (4). Therapeutic trials in mice given oral or intraperitoneal isoprinosine 24 h after challenge with A(H_2N_2)/Bethesda/10/63 again showed delayed or altered mortality at a low dose (4 LD_{50}) only, and significant protection was afforded only when the drug was continued for 10 days.

In contrast to the antiviral activities, no analgesic or antipyretic properties have been attributed to isoprinosine in animal models, and there appears to be no action on the vasomotor reactions to acetylcholine, histamine, and serotonin.

In the present study, the antiviral activities of isoprinosine demonstrable in animal models did not appear to protect human volunteers from artificial challenge with A(H_3N_2)/HK influenza virus. Clinical illness was not delayed in onset, lessened in severity, nor shortened in course. Slight differences in temperature elevation were not statistically significant. The only demonstrable beneficial effect of the drug was that the total number of virus isolates was slightly but significantly less in the isoprinosine-treated group. The acquisition of neutralizing antibody titers was not inhibited by drug therapy and was similar for treated and control groups. Side effects were limited to a temporary elevation of serum uric acid.

Although isoprinosine appears to offer no protection from nor alleviation of clinical illness associated with A(H_3N_2)/HK influenza virus, it could be of epidemiological significance in decreasing virus shedding in epidemics.

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