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# Oral LY217896 for Prevention of Experimental Influenza A Virus Infection and Illness in Humans

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**The efficacy and safety of oral LY217896 for prevention of experimental influenza A/Kawasaki/86 (H1N1) virus infection were assessed in susceptible males randomly assigned to receive LY217896 (75 mg) or placebo once daily for 7 days beginning 24 h prior to viral challenge. The rates of virus shedding (100% in both groups), days of viral shedding (3.1 ± 1.3 for the LY217896 group; 2.8 ± 1.3 for the placebo group), and titers of virus in nasal washings did not differ between the groups. Mild upper respiratory tract illness (72% in the LY217896 group; 69% in the placebo group) developed in similar proportions of each group. LY217896 was associated with asymptomatic rises in serum uric acid levels and was ineffective in modifying the virologic or clinical course of experimental influenza A (H1N1) virus infection.**

A continuing need exists for anti-influenza agents effective in the treatment and prevention of influenza A and B virus infections. The compound LY217896 (1,3,4-thiadiazole-2-ylcyanamide) is a water-soluble thiadiazole derivative which possesses broad antiviral activity in vitro and in animal models against orthomyxo- and paramyxoviruses (3, 5, 7). When administered orally, intraperitoneally, or by aerosol, LY217896 significantly reduced pulmonary viral titers and protected mice against lethal influenza A or B virus challenge (3). Single intraperitoneal doses of LY217896 were effective in suppressing fever induced by influenza B virus infection of ferrets (3), and low intraperitoneal doses (3 mg/kg of body weight per day) were effective in reducing lung viral titers following respiratory syncytial virus or parainfluenza virus type 3 infection of cotton rats (7).

In phase I studies with healthy volunteers, single doses of up to 500 mg were well tolerated and had predictable pharmacokinetics (1). The maximum concentration in serum averages 0.7 μg/ml at a dose of 17 mg and 32.2 μg/ml at 500 mg, and the plasma drug elimination half-life is about 2 h (1). An initial human trial, in which doses of 50 mg once daily were begun 24 h after challenge with influenza B/Yamagata/88 virus, found that LY217896 administration was not associated with antiviral effects or a reduction in illness (2). Consequently, the current clinical trial was conducted to determine the prophylactic efficacy of a higher dose of oral LY217896 in experimental influenza A (H1N1) virus infection.

**Study design.** Healthy males age 18 to 45 years with titers of hemagglutination inhibition (HI) antibody to the challenge virus of ≤1:8 were recruited for participation. All provided written, informed consent in a form approved by the Institutional Review Boards of the respective institutions and were compensated for participation. Volunteers were confined in an isolation facility for a period of 8 to 9 days during the study as described previously (6). After an 1-day observation period, subjects were randomly assigned to treatment with oral LY217896 (75 mg per dose) or placebo once each morning for 7 days. After the second drug dose (i.e., 24 h after initiating treatment), they were inoculated intranasally with approximately $10^7$ 50% tissue culture infective doses of safety-tested influenza A/Kawasaki/86 (H1N1) virus (0.25 ml per nostril) provided by L. Potash (PRI/DYN Corp., Rockville, Md.).

Daily nasal washings beginning 1 day before virus inoculation were inoculated onto monolayers of Madin-Darby canine kidney (MDCK) cells for isolation of influenza virus by standard techniques. For samples which were positive for virus, once-frozen aliquots (−70°C) were thawed and serial $\log_{10}$ dilutions were titered in quadruplicate monolayers of MDCK cells. Sera obtained 1 day before virus challenge and approximately 3 weeks after challenge were assayed in parallel to determine titers of HI antibody to the challenge virus.

Beginning prior to virus inoculation, each volunteer rated the severity (4-point scale, absent to severe) of 10 symptoms twice daily, in the morning and the late afternoon (6). Subjects had daily standardized physical assessments by a physician, and oral temperatures were measured four times daily. Fever was defined as the occurrence of an oral temperature of 37.8°C or higher, and administration of acetaminophen was allowed for fever or disabling symptoms. Safety monitoring included routine hematology, blood chemistries, and urinalysis studies done before virus challenge, every other day during the treatment period, and approximately 3 weeks after cessation of therapy. Stool samples were tested for occult blood during drug administration.

**Pharmacokinetic studies.** The pharmacokinetic profile of LY217896 during the course of experimental influenza virus infection was evaluated by obtaining samples for plasma drug concentrations once each day at designated intervals following dosing. On study days 1 to 7 the interval from the last dose to time of drug sampling was planned to be 0.5, 10.0, 2.0, 8.0, 12.0, 4.0, and 1.0 h, respectively. Plasma samples were frozen at −20°C for later analysis by Albert Peyton at Lilly Research Laboratories. The lower limit of detection of this assay is 100 ng/ml.

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TABLE 1. Infection and illness occurrence in volunteers challenged with influenza A/Kawasaki/86 (H1N1) virus

| Treatment group (*n*) | % of subjects with: | | | | | | Mucus wt[a] (mean ± SD g/6 days) |
|---|---|---|---|---|---|---|---|
| | Virus shedding | Seroconversion[b] | Upper respiratory illness[c] | Cough for ≥2 days | Myalgia | Fever[d] | |
| LY217896 (18) | 100 | 83 | 72 | 11 | 44 | 11 | 13.6 ± 22.9 |
| Placebo (16) | 100 | 81 | 69 | 13 | 44 | 6 | 16.4 ± 20.5 |

[a] Based on results for 10 LY217896-treated and 12 placebo-treated subjects studied at University of Virginia.
[b] Fourfold or greater rise in HI antibody titers at 3 weeks postinoculation.
[c] Coryza and/or pharyngitis for ≥2 days.
[d] Oral temperature of ≥37.8°C.

**Infection and illness rates.** Thirty-six subjects were randomly allocated to treatment, 19 to the LY217896 group and 17 to the placebo group. One LY217896 recipient had an acute-phase HI antibody titer of 1:16 and was not considered evaluable. One placebo recipient was removed from the study after only one dose and before virus inoculation because of elevated serum transaminase concentrations. Consequently, 18 LY217896-treated and 16 placebo-treated subjects were considered evaluable for drug efficacy. All the participating subjects were male, and the mean ages were 22.0 years in the LY217896 group and 21.6 years in the placebo group. The groups were also comparable in regard to average height (181 ± 6 versus 178 ± 10 cm), weight (80.6 ± 10.9 versus 84.7 ± 14.3 kg), and ethnicity. Acetaminophen was used by one LY217896 recipient on 1 day and three placebo recipients on a total of 4 study days.

All evaluable subjects in the LY217896 and placebo groups had virus recovered from respiratory secretions on at least 1 day during the period of confinement (Table 1). The proportion of subjects shedding virus on each of the postinoculation days was comparable in the two groups (data not shown). The mean durations of virus shedding ± standard deviation were similar in the LY217896 (3.1 ± 1.3 days) and placebo (2.8 ± 1.3 days) groups. Virus titers peaked 24 to 48 h after inoculation, and no differences in titers were evident between the groups on any of the postinoculation days (Fig. 1). Similarly, the proportions of subjects with a fourfold or greater rise in HI antibody titers at follow-up were comparable (81 to 83%) in the two groups. The observed changes in HI antibody titers were also comparable in the LY217896 (mean ± standard deviation $\log_2$, 2.7 ± 1.0) and placebo (2.3 ± 1.2) groups.

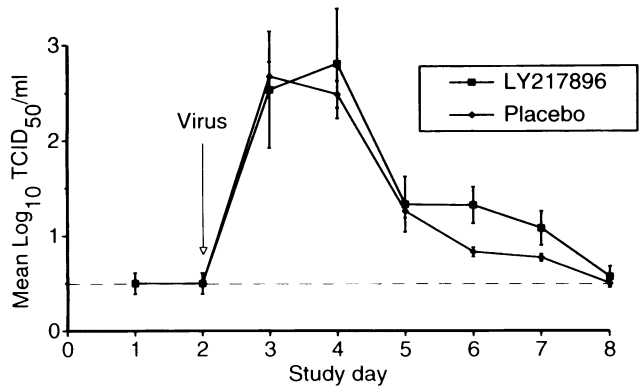


FIG. 1. Quantitative virus shedding patterns following experimental influenza A/Kawasaki/86 (H1N1) virus challenge in volunteers treated with LY217896 or placebo. Nasal wash titers were determined in MDCK monolayers. Culture-negative samples were assigned a value of 0.5 $\log_{10}$ 50% tissue culture infective dose ($TCID_{50}$)/ml, the lower limit of assay sensitivity (dotted line), for calculation purposes. The values are listed as mean ± standard error of the mean.

Although nearly half of the subjects in each of the groups reported generalized myalgias on at least one postchallenge day, the occurrence of objectively documented fever was infrequent in both groups (Table 1). Approximately two-thirds of the subjects in each group experienced upper respiratory tract illness on at least two consecutive days, but fewer than 15% in each group had a cough persisting for this period of time (Table 1). No differences between the groups were found in the weights of expelled nasal mucus (Table 1).

Digital tympanometry (4) detected abnormalities in middle-ear pressures frequently during experimental influenza virus infection in both groups. All 12 placebo recipients (100%) and 7 of 10 LY217896 recipients (70%) had abnormal middle-ear pressures ($< -50$ or $> +20$ mm $H_2O$) documented on at least 1 postchallenge day. Bilateral major underpressures ($\leq -100$ mm $H_2O$) were documented in 33% of placebo-treated and 30% of LY217896-treated volunteers during the postchallenge period. Only two subjects in each group reported ear pain, but otoscopic examination detected erythema of the tympanic membrane on two or more days in 42% of placebo-treated and 50% of LY217896-treated subjects, including one subject who was later treated with antibiotics for acute otitis media.

**Tolerance.** None of the subjects discontinued the study drug prematurely as a result of an adverse event. Nausea was reported by one LY217896 recipient, but none of the subjects experienced diarrhea. No laboratory changes of clinical importance were observed, with the exception of serum uric acid. LY217896-treated subjects had significant rises in serum uric acid concentrations by the completion of the treatment phase of the study (mean 12% rise) ($P < 0.01$, pretherapy versus posttherapy), compared with a mean 9% decrease in serum uric acid in the placebo group ($P < 0.001$ versus LY217896 group). The increases observed in the LY217896 group were within the normal range of laboratory values, were not associated with symptoms, and resolved promptly after cessation of drug administration.

**Plasma LY217896 concentrations.** In the subset of volunteers at the University of Virginia, plasma samples were collected before drug administration and on seven subsequent days at designated intervals after a preceding dose (Fig. 2). Peak concentrations in plasma averaged approximately 3.8 μg/ml at approximately 1 h after dosing in the LY217896 recipients. Concentrations began declining in a log-linear fashion by approximately 4 h postdosing and were below the limits of assay detection in most subjects by 10 to 12 h after dosing. The half-life based on time relative to the dose was ~2.4 h, consistent with that previously found in healthy volunteers (1).

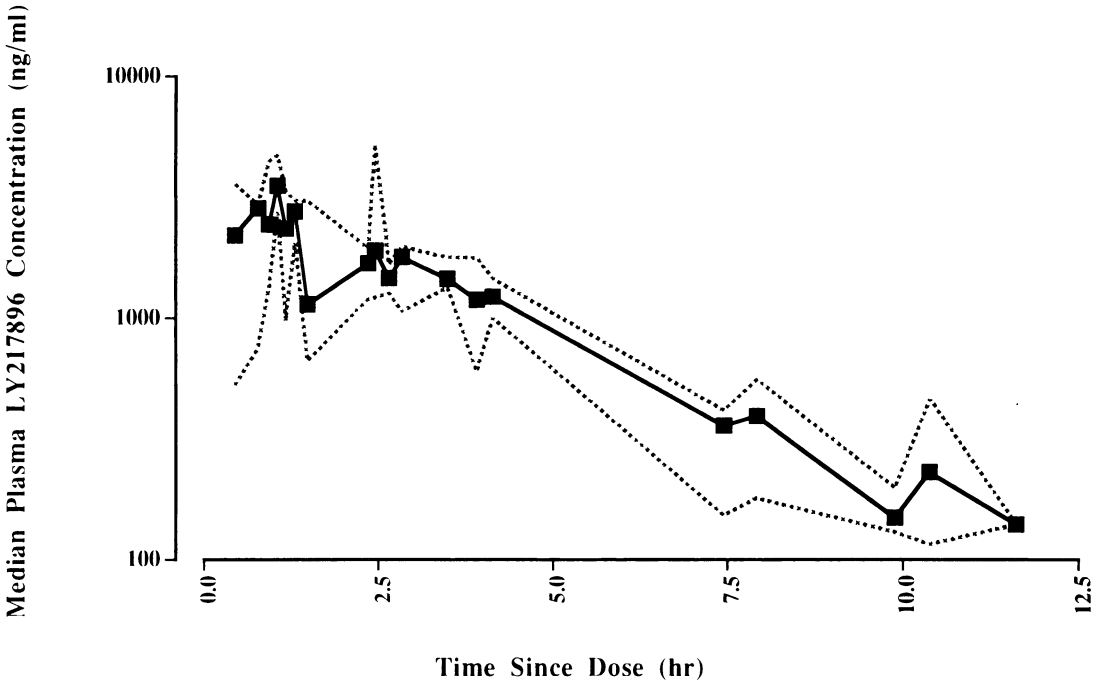


FIG. 2. Plasma LY217896 concentrations after 75-mg daily doses. The solid line represents the median value for 12 subjects. The dashed lines represent the minimum and maximum concentrations in plasma obtained for each time point. The lower limit of assay sensitivity was 100 ng/ml.

**Comment.** The current study found that oral administration of LY217896 at a dose of 75 mg/day did not significantly alter the virologic or clinical course of experimentally induced influenza A/Kawasaki/86 (H1N1) virus infection. Specifically, oral LY217896 did not decrease the frequency, duration, or quantity of virus shedding or prevent the development of respiratory tract symptoms related to influenza. Under in vitro conditions, LY217896 concentrations of approximately 0.4 to 1.5 μg/ml inhibit plaque formation by a variety of influenza A and B viruses in MDCK cells (3). In yield reduction assays, concentrations of 0.1 to 1.0 μg/ml inhibit the replication of influenza A virus in MDCK or primary rhesus monkey kidney cell cultures (5). Although the subjects in the current study achieved peak plasma LY217896 concentrations which exceeded such in vitro inhibitory concentrations by severalfold, we did not observe significant antiviral effects.

This lack of an antiviral effect could have been related to various factors, including the relatively short half-life of LY217896 in plasma in humans and the need for more frequent administration, inadequate distribution to the respiratory tract mucosa, and/or inadequate intracellular uptake or metabolism to an active form. The dose regimen selected for this trial was based on the results of animal model studies and of phase I safety studies in humans. Infrequent dosing (approximately twice daily) by intraperitoneal or oral routes was found to be protective in experimental murine models of influenza (3). Similarly, once-daily intraperitoneal administration of low doses (3 mg/kg/day) reduced replication of either respiratory syncytial virus or parainfluenza virus type 3 in cotton rats (7), although 10-fold-higher doses were not active after oral administration.

The dose of LY217896 used in this study was well tolerated during short-term administration. In contrast to anecdotal reports of gastrointestinal intolerance during phase I trials, it did not cause diarrhea or gastrointestinal upset. Except for one LY217896 recipient who had guaiac-positive stools on several treatment days, no detectable blood loss in stool samples was found (data not shown). In addition, no adverse effects on formed blood elements, which might suggest depression of bone marrow function, were observed. The only laboratory abnormality consistently associated with LY217896 administration was increased serum uric acid during the treatment period. Although asymptomatic and usually remaining within the normal range of values, these increases were observed in most volunteers and were possibly related to either decreased clearance of uric acid at the renal level or increased production. Preliminary studies of urinary excretion of uric acid in uninfected volunteers have found evidence for increased production, likely indicating more rapid turnover of dividing cells. The uricogenic effect is dose related and has been described previously for patients receiving thiadiazoles.

In summary, the finding of adverse effects on uric acid metabolism at doses that are not associated with antiviral effects suggests that oral LY217896 is unlikely to have clinically useful anti-influenza virus activity.

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