Effect of Rimantadine Treatment on Clinical Manifestations and Otologic Complications in Adults Experimentally Infected with Influenza A (H1N1) Virus

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Susceptible adults (n = 105) were enrolled into a randomized double-blind study of rimantadine treatment of experimental influenza A infection. Subjects were cloistered for 8 days and challenged with a rimantadine-sensitive strain of influenza A H1N1 virus at the end of the first day. Forty-eight hours after challenge and for 8 days, 54 subjects received placebo and 51 received rimantadine (100 mg orally, twice a day). Symptoms, signs, and pathophysiologies were monitored. Nine subjects were not infected. Seventeen subjects (38%) in the rimantadine and 26 (53%) in the placebo group became ill. A beneficial effect of rimantadine was documented for virus shedding, symptom load, and sinus pain. Rimantadine had no effect on nasal patency, mucociliary clearance, nasal signs, or on symptoms and signs of otologic complications. These results do not support a preventive effect of rimantadine on the development of otologic manifestations of influenza A infection in adults.

Otitis media (OM) is characterized by the presence of inflammation within the middle ear and is second in frequency of diseases affecting infants and children only to upper respiratory tract infections (URIs) [1]. Established OM is refractory to current methods of medical treatment and can persist for weeks, months, or even years [2]. Epidemiologic studies show that >50% of new episodes of OM are temporally associated with a viral URI, and experimental studies document a causal relationship between the two diseases [3–8]. Our understanding of OM pathogenesis predicts a temporal delay between the onset of virus infection, symptom presentation, and OM. In theory, this delay can be exploited to reduce the risk of otologic complications by treating the primary virus infection. This strategy has advantages over the prolonged use of antivirals for prophylaxis. Since the target population is circumscribed to symptomatic “at risk” persons, the required duration of therapy can be limited to the expected period of viral shedding, and an antiviral with a reasonable likelihood of efficacy can be selected on the basis of concurrent viral activity within the community or by rapid diagnostic testing. Thus, a significant effect on the incidence and prevalence of OM could be realized by targeted antiviral treatment of seasonally defined URIs.

Experimental respiratory virus infection of human adults has been used to study the pathogenesis of the virus infection and its complications. In two studies, experimental influenza A virus infection caused sequential otologic complications, including eustachian tube dysfunction (80%), middle ear underpressures (70%), and OM (20%) after the initial expression of the signs and symptoms of the primary infection [7, 8]. These observations suggest a causal pathway leading from virus infection to OM via the intermediate development of eustachian tube dysfunction and middle ear underpressures. This experimental setting may be ideal for evaluating strategies to prevent the otologic complications of a viral URI, since objective markers of otologic involvement are provoked in relatively high number, there appears to be a window for prophylaxis of the complication by antiviral treatment after symptom presentation, and an approved relatively safe and effective antiviral, rimantadine, is available for treatment [9–11]. The aim of the present study was to determine if rimantadine treatment after symptom onset decreases the frequency and severity of the otologic complications during experimental influenza A virus infection in adults.

Materials and Methods

Population. Adult subjects (ages 18–50 years) were recruited by advertisement and screened for susceptibility by assay of serum hemagglutination-inhibition (HI) antibodies to influenza A/Kawasaki/86 H1N1 [12]. Subjects were excluded from the study if they had a history of nasal or otologic surgery, pulmonary, cardiovascular, renal, or other serious disease or seizures, or had a symptomatic URI within the prior 30 days, OM within the last 6 months, concurrent pregnancy, nasal or otologic signs and symptoms, abnormal clinical profiles, seropositivity for human immunodeficiency virus, or an HI antibody titer >1:10.

Medications. Study medication was rimantadine HCl (Flumadine, 100-mg tablets; Forest Pharmaceuticals, St. Louis). Indi-
vial doses with lactose filler were repackaged in gelatin capsules, and identical-appearing lactose placebos were formulated at the Children's Hospital of Pittsburgh. Randomization codes were generated and maintained under seal until completion of the study. Study medication was supplied in numerically coded containers and dispensed under observation as unit doses at the cloister site.

**Challenge virus.** The challenge virus was a safety-tested, rimantadine-sensitive clinical isolate of influenza A/H1N1 virus (wild type, lot E-262; NIH) [11].

**Design.** The study was a double-blind, placebo-controlled randomized trial of rimantadine treatment of experimental influenza A virus infection. Subjects were randomized by entry number (equal numbers for blocks of 10) to treatment with either placebo or rimantadine and studied in 3 cohorts (n = 30, 32, and 43) during 1 calendar year. Subjects reported to a local hotel at ~6:00 p.m., a time that was defined as the beginning of study day 0. They were cloistered in individual rooms for 8 days (study days 0–7). Twenty-four hours after admission, 0.25 mL of virus inoculum per nostril was administered intranasally as coarse drops (total dose, 10^7 TCID50). Forty-eight hours after inoculation, subjects were given under observation their first dose of medication as per code (100 mg of rimantadine HCl or placebo as capsules by mouth). Repeat dosing was done at 12-h intervals for a total of 8 days (5 days of cloister and 3 outpatient days). Subjects were not permitted to take over-the-counter or prescription medications with the exception of birth control pills.

Each day each subject received a general physical examination by a physician, pneumatic otoendoscopical and nasal examinations by an otolaryngologist, and nasal lavage for virus recovery as previously described [13]. Symptoms consisting of sneezing, nasal discharge, nasal congestion, earache, sinus pain, sore throat, cough, chest congestion, malaise, headache, chilliness, muscle ache, joint pain, sweats, and fever were rated by the subjects on a four-point, 0 to 3 scale, corresponding to none, mild, moderate, or severe. Beginning on study day 3, the subjects completed a second daily symptom diary, which listed 15 symptoms reported previously as being possible or probably related to either amantadine or rimantadine treatment. Also, on each day of cloister, expelled nasal secretion weights were determined, nasal mucociliary clearance function was evaluated (1 time), nasal congestion was measured by anterior rhinometry (2 times), and temperatures, middle ear pressures, and eustachian tube function were measured (3 times) using instruments and methods previously described [7].

All subjects developing OM were treated with a 10-day course of an appropriate antibiotic. Collected lavage samples were inoculated immediately into veal infusion broth and frozen at ~70°C. These were inoculated onto MDCK monolayers in triplicate, and virus was identified by hemadsorption [14]. The first isolate was confirmed to be influenza A by immunofluorescence testing. Convalescent blood samples were collected ~2 weeks after subjects were dismissed from cloister and assayed for HI antibody titer by standard techniques [12].

**Study data.** Data consisted of the results for viral shedding, vital signs, subject-rated symptoms, physician-rated signs, secretion weights, and tests measuring nasal mucociliary clearance function, nasal patency, eustachian tube function, and middle ear pressure. The duration of viral shedding was defined as the number of days between the first and last day of viral isolation. Infection was defined as viral shedding on ⩾1 day or a ⩾4-fold rise in specific-serum HI antibody between the prechallenge and convalescent samples. Illness was defined as a baseline-adjusted symptom score of ⩾4 on 2 consecutive days.

Data collected on study day 0 were considered to be representative of the baseline state, and the values for all continuous variables on study days 1–7 were adjusted by subtracting the corresponding value recorded on study day 0 (baseline-adjusted). For each subject and study day, four influenza-related summary symptom scores were constructed corresponding to nasal symptoms (sum of baseline-adjusted scores for rhinorrhea, nasal congestion, and sneezing), throat symptoms (sum of baseline-adjusted scores for sore throat and cough), systemic symptoms (sum of baseline-adjusted scores for malaise, headache, chilliness, muscle ache, joint pain, sweats, and fever), and total symptoms (sum of all scores).

For each subject on each day, symptoms suggestive of complications including earache, sinus pain, and chest congestion were reduced to a dichotomous classification (present or absent). For treatment side effects, the symptom was defined as present or absent for each subject on the basis of a score >0 on any treatment day. Physician-rated nasal and otologic signs were reduced to a dichotomous normal-abnormal classification. Similarly, subjects were classified dichotomously with respect to the presence or absence of OM on the basis of a diagnosis of unilateral or bilateral effusion without respect to accompanying symptoms. For each ear and study day, middle ear pressure was defined as abnormal if the measured pressure was less than or equal to ~100 mm H2O. Eustachian tube function was defined as abnormal if, at a given test session, sonotubometry failed to detect tubal openings (>10 dB increase in canal sound pressure level) on any of 4 consecutive deglutions.

**Statistical methods.** In the analysis, the data for the 3 cohorts were combined. The tested hypothesis was that active treatment would promote a more rapid resolution of signs, symptoms, and pathophysiologies compared with that of placebo treatment, and therefore one-tailed statistical tests were used. For global tests of patterned responses in continuous variables, we used repeated measures analysis of variance (ANOVA) (version 3.1, Stat Soft, Tulsa, OK). A significance level of α ⩽ .10 was considered sufficient to allow for secondary testings of the paired data for each day using the Mann-Whitney U or Student’s t test as appropriate (α ⩽ .05). For global tests of population responses in dichotomous variables, Fisher’s exact test was used and evaluated at α ⩽ .05 (version 6.0, NCSS, http://www.ncss.com). For repeated measures in dichotomous variables, Fisher’s exact test was evaluated at α ⩽ .03 to compensate for the effect of the multiple comparisons. The convention mean ± SE was used throughout.

**Results.**

**Population.** We studied 105 susceptible subjects (1 Hispanic, 18 black, and 86 white). Mean age was 31.1 ± 10.6 years (range: 18–50). There were 50 men. There were 2 dropouts: 1 subject assigned to the placebo group left cloister and 1 assigned to the rimantadine group refused treatment after dose one. Of those remaining, 50 received rimantadine treatment and 53 were given placebo.

**Side effects of medications.** None of the placebo-treated subjects reported adverse events. One rimantadine-treated sub-
ject discontinued the medication after the first dose because of self-reported hallucinations, confusion, and nervousness; a second subject discontinued treatment after the ninth dose because of gastrointestinal complaints. More specific side effects were reported by a greater percentage of subjects receiving the rimantadine treatment than by those given placebo: dry mouth (active, 24%; placebo, 4%), anxiety (active, 14%; placebo, 7%), nervousness (active, 6%; placebo, 2%), shakiness (active, 4%; placebo, 2%), nausea (active, 10%; placebo, 6%), vomiting (active, 4%; placebo, 0%), and hallucinations (active, 2%; placebo, 0%); however, only the between-group difference for dry mouth was significant ($P < .05$).

**Infection and illness.** A total of 35 subjects (70%) in the rimantadine and 42 (79%) in the placebo group shed virus, and 36 (72%) in the rimantadine and 46 (87%) in the placebo group seroconverted. The average days of virus shedding was 1.8 ± 1.6 for the rimantadine group and 3.2 ± 2.3 for the placebo group overall and 2.6 ± 1.3 for the rimantadine-treated and 4.0 ± 1.8 for the placebo-treated subgroups who shed virus ($P < .05$). Nine subjects were not infected (5, rimantadine; 4, placebo). All comparisons are based on data available for the infected subgroups of the rimantadine- (n, 45) and placebo-treated (n = 49) subjects.

The first observation of shedding occurred before initiation of treatment in all (31, day 1; 4, day 2) of the rimantadine-treated subjects and in 95% (33, day 1; 7, day 2; 2, day 3) of the placebo-treated subjects. The frequencies of viral shedding on each day were 69%, 73%, 33%, 11%, 7%, 4%, and 2% for the rimantadine group and 67%, 78%, 65%, 57%, 45%, 24%, and 4% for the placebo group. Between-group differences were statistically significant on study days 3, 4, 5, and 6 ($P < .03$).

The illness caused by the virus infection was relatively mild. Only 3 subjects (5%) in the rimantadine group (before treatment initiation) and no subject in the placebo group had significant temperature elevations ($\geq 37.8^\circ C$). A total of 17 subjects (38%) in the rimantadine group and 26 (53%) in the placebo group were judged to be ill ($P = \text{not significant [NS]}$). The baseline-adjusted total symptom scores for the follow-up period varied between 0 and 65; 25% of the subjects had a total score of $\leq 6$ in the rimantadine group and of $\leq 4$ in the placebo group.

**Symptoms and signs of influenza illness.** Figure 1 shows the average baseline-adjusted, nasal, throat, and systemic symptom scores as function of study day for rimantadine-treated subjects (open bars) and upper and lower bounds of 95% confidence interval about average values for placebo-treated subjects (○). Significance levels of treatment effect were determined by analysis of variance: $P = .05$ for total, $P = .06$ for systemic and for throat, and $P = .32$ for nasal symptom summary score.

Figure 2 shows the daily percentages of infected subjects in the 2 groups presenting with a total score of $\geq 4$ and the percentages of subjects in the 2 groups who reported sinus pain and chest congestion. The frequencies of symptom reporting for these three measures showed a more pronounced decrease after initiation of the rimantadine treatment compared with the placebo treatment. Between-group differences were significant on days 4 and 5 for total symptoms and on day 5 for sinus pain ($P < .03$). On day 0, the frequency of subjects presenting with abnormal physician-rated signs for nasal patency (active, 35%; placebo, 18%), mucosal edema (active, 26%; placebo, 10%), mucosal color (active, 24%; placebo, 10%), and quantity of rhinorrhea (active, 33%; placebo, 14%) was greater in the rimantadine group. Those frequencies increased significantly in both groups to peak between days 3 and 5 and then slowly decreased over the follow-up period. Between-group differences at baseline were retained throughout the study period with no evidence of an effect of the active treatment on any of these measures. No subjects had signs of sinus drainage.
brane signs in both groups peaked at ~50% on day 3 and then decreased. There was no evidence of treatment effect on either of these measures ($P = \text{NS}$).

On each study day, the number of sessions with eustachian tube function failure was summed over the two ears, yielding a maximum value of 6 (2 ears $\times$ 3 sessions/day) for this index of dysfunction. For both groups, the index showed a progressive increase to a maximum on day 3 and then a partial recovery with time. On day 0, the average value of the index was $0.6 \pm 0.9$ for the placebo group and $1.3 \pm 1.8$ for the rimantadine group. The average change from baseline in this index during the treatment period was $1.3 \pm 1.9$ and $1.4 \pm 2.1$ for the rimantadine and placebo groups, respectively ($P = \text{NS}$). Figure 3 shows the percentage of persons in the 2 groups with at least unilateral abnormal middle ear pressure on each day. For both groups, that frequency increased on study day 2, peaked on study day 4 or 5, and showed little evidence of recovery. At most times, the percentage of persons with abnormal middle ear pressures was greater in the rimantadine group. For ears, the average number of days with abnormal negative middle ear pressure was greater in the rimantadine group. For ears, the average number of days with abnormal negative middle ear pressure was greater in the rimantadine group.

Figure 2. Daily frequencies of subjects in rimantadine (■) and placebo (◇) treatment groups who reported a total symptom score $\geq 4$, sinus pain, and chest congestion (CONG).

In both groups, temperature and secretion weights peaked on study day 2 or 3 and then decreased to approach baseline. Average nasal conductance showed a shallow and progressive decrease with time, and average clearance time increased to a plateau maintained over days 4–7. The significance of the treatment effect calculated by ANOVA was $P = .67$ for conductance, $P = .19$ for temperature, $P = .35$ for clearance, and $P = .12$ for secretion weight. The average change from baseline summed over the treatment period was $0.1 \pm 5.7$ and $1.6 \pm 5.4^\circ\text{C}$ for temperature, $1.4 \pm 10.3$ and $6.0 \pm 17.0$ g for secretion weight, $15.3 \pm 32.0$ versus $14.6 \pm 29.1$ min for clearance time, and $-0.24 \pm 0.74$ versus $-0.30 \pm 0.65$ for conductance in the rimantadine and placebo groups, respectively ($P = \text{NS}$).

Otolologic signs, symptoms, and pathophysiologies. Figure 3 shows data on the temporal expression of otologic symptoms, signs, and pathophysiologies for the 2 groups. A total of 16 subjects (36%) in the rimantadine group and 26 (58%) in the placebo group reported earache on at least 1 day of cloister. For both groups, the frequency of subjects reporting earache increased to peak at ~30% on day 3 and then decreased to ~10%. The number of persons with abnormal tympanic membrane signs and abnormal middle ear pressure (ABN MEP) by tympanometry.

Figure 3. Daily frequency of subjects in the active (■) and placebo (◇) treatment groups who reported earache and had abnormal tympanic membrane signs and abnormal middle ear pressure (ABN MEP) by tympanometry.
ear pressures during the treatment period was 1.6 ± 1.9 in those given rimantadine and 1.6 ± 1.8 in those given placebo \( (P = \text{NS}) \). For persons, the average number of days with abnormal middle ear pressures during the treatment period was not significantly different in the rimantadine (2.4 ± 2.0) and placebo (2.0 ± 2.0) groups.

During the study, 5 subjects (6%) were diagnosed with OM. Four cases were unilateral with onsets on study days 1 (rimantadine), 3 (rimantadine), 4 (placebo), and 6 (placebo); 1 case was bilateral (rimantadine) with an onset on study day 4 \( (P = \text{NS}) \). Tympanocentesis was done on 2 of these ears, and the recovered effusions were culture-negative for bacteria and virus.

**Discussion**

These results confirm the previously reported benefit of rimantadine treatment for certain signs and symptoms of influenza A virus infection in adults [9, 10]. Rimantadine given orally at a dose of 100 mg twice daily beginning 48 h after virus exposure and at the time of peak symptoms shortened the duration of virus shedding and significantly decreased the total symptom load. Also, there were significantly fewer infected subjects who reported sinus pain, and fewer reported chest congestion in the group given rimantadine. No effect of rimantadine treatment was suggested by the objective tests that assessed nasal patency and nasal mucociliary clearance function or by the physician-rated signs of nasal inflammation. As previously reported, rimantadine treatment did not adversely affect the immune response to influenza A virus as evidenced by the similar frequency of subjects in the 2 groups who developed increased homologous HI serum antibody titers after challenge [15].

A variety of evidence suggests that the development of OM as a complication of viral URIs is mediated by a virus-induced disruption of normal eustachian tube function, which in turn causes middle ear underpressures and OM by hydrops ex vacuo [7, 8]. Thus, treating a URI episode with an effective antiviral could prevent the development of OM by decreasing the virus load and suppressing or reversing the provoked eustachian tube dysfunction and consequent middle ear abnormalities. The results of the present study for otologic symptoms, signs, and pathophysiology do not support that hypothesis. Specifically, there were no significant or apparent differences among treatment groups in the frequency of infected subjects reporting earache, in the physician-rated signs of otologic involvement, in the frequencies of subjects with either eustachian tube dysfunction or middle ear underpressures, or in the number of diagnosed OM episodes. Of interest, in the placebo-treatment group, the symptoms and signs of influenza illness were relatively mild compared with those of previous studies, but the frequencies of otologic symptoms and signs, including earache and abnormal middle ear pressures, were similar across studies [7, 8, 16]. Also of note was the early development of these indicators of otologic complications vis-à-vis symptom presentation, which had the effect of decreasing the window available for prophylaxis. This temporal pattern is not consistent with that reported for other studies using this model and, if confirmed, would undermine the rationale for this intervention strategy [7, 8, 16].

These results suggest the hypothesis that the different influenza A–provoked signs, symptoms, and pathophysiologies can be assigned to one of two domains on the basis of a positive response to rimantadine treatment. The outcomes positively affected by rimantadine are associated with measures of viral replication, secretion production, and generalized signs and symptoms. These outcomes are the expressed effects of the primary host response to infection and may be feedback-controlled by the decreased virus load consequent to rimantadine treatment [17, 18]. In contrast, the domain of responses not affected by rimantadine include nasal congestion, mucociliary stasis, and eustachian tube obstruction. These responses are provoked by a variety of stimuli that promote nasal inflammation and may function to protect the sinuses, lungs, and middle ears [19]. Consequently, they may be feedback-modulated by factors that track the degree of nasal inflammation, residual effects of which can be measured long after suppression of the inciting stimulus. Unfortunately, the continued persistence of these responses can be pathophysiological and, by failing to resolve promptly, can in themselves promote the development of complications. If validated, effective prophylaxis of middle ear complications during a viral URI may require both antiviral therapy and adjunctive antiinflammatory therapy.

In the current study, the initiation of treatment was timed to correspond to that of unambiguous symptom presentation, since in practice, antiviral treatments would be initiated only after recognition of the presence of a URI by symptoms or signs and subsequent determination of an etiology potentially responsive to the specific antiviral (e.g., seasonal virus epidemic, rapid virus identification test). Also, the choice to explore the efficacy of antiviral treatment as opposed to antiviral prophylaxis was made after consideration was given to the overall welfare of the intended target population, children. To have a significant effect on the incidence of OM, rimantadine prophylaxis would need to be extended to a majority of infants and children at risk for influenza A infection. While rare, the side effects of rimantadine would be made more manifest in frequency by the large number of children targeted for prophylaxis. The probability of realizing these side effects could be lessened by decreasing rimantadine use to limited periods of time in children with a high likelihood of concurrent infection.

However, it is likely that the potency of the antiviral and timing of the intervention are important variables in developing strategies to prevent OM secondary to viral URIs. In that regard, newer drugs, such as intranasal zanamivir (GG167), a viral neuraminidase inhibitor with significantly lesser side effects than rimantadine, can be considered for extended prophylaxis or early treatment of influenza virus infection to prevent...
In support of this, a recent study using experimental influenza A infection of adult volunteers showed that zanamivir prophylaxis and early treatment (26–32 h after virus inoculation) significantly reduced the frequencies of earache and abnormal middle ear pressures of infected subjects compared with placebo treatment [16]. These promising results suggest that our goal of preventing OM by intervening in the course of a viral URI may be achievable with the advent of less toxic and more potent antiviral agents.

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**References**