A CONTROLLED TRIAL OF AMANTADINE AND RIMANTADINE IN THE PROPHYLAXIS OF INFLUENZA A INFECTION

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Abstract Four hundred fifty volunteers participated in a placebo-controlled, double-blind, randomized trial of the prophylactic effects of rimantadine and amantadine during an outbreak of influenza A. The subjects received drugs orally at a dose of 100 mg twice a day for six weeks.

Influenza-like illness occurred in 41 per cent of the subjects receiving placebo but in only 14 per cent of those receiving rimantadine and 9 per cent of those receiving amantadine ($P<0.001$ for either drug vs. placebo). Laboratory-documented influenza occurred in 21 per cent of placebo recipients, 3 per cent of rimantadine recipients, and 2 per cent of amantadine recipients ($P<0.001$). These findings represent efficacy rates of 85 per cent for rimantadine and 91 per cent for amantadine, as compared with placebo. More recipients of amantadine (13 per cent) than recipients of rimantadine (6 per cent; $P<0.05$) or placebo (4 per cent; $P<0.01$) withdrew from the study because of central-nervous-system side effects. On the basis of this study, rimantadine appears to be the drug of choice for the prophylaxis of influenza A. (N Engl J Med. 1982; 307: 580-4.)

AMANTADINE hydrochloride (1-adamantanamine hydrochloride) was licensed in 1966 for the prophylaxis of infections with influenza A viruses of the H2N2 subtype, and it has subsequently been approved for the prophylaxis of infections with all influenza A subtypes. Despite accumulated evidence of the efficacy of amantadine in the prophylaxis of influenza in human beings,[1-6] the drug has received relatively little use for this purpose. In part, this has been a result of continued challenges to the evidence of its efficacy by some workers,[7] as well as concern over its reported side effects, which have varied considerably in frequency from study to study.[8,9]

Rimantadine hydrochloride (α-methyl-1-adamantanemethylamine hydrochloride) is an amantadine analogue that has been reported to be more active than amantadine against influenza A viruses in vitro[10] and against experimentally induced influenza A infection in laboratory animals.[11] On the basis of experience in the Soviet Union, where this agent is widely employed, Soviet investigators have suggested that rimantadine may be more effective and perhaps better tolerated than amantadine.[12] However, rigorously controlled field trials demonstrating the efficacy of rimantadine in the prophylaxis of influenza A infection have not been reported.

An outbreak of influenza A in Burlington, Vermont, provided an opportunity for us to compare amantadine and rimantadine in a placebo-controlled, double-blind, randomized chemoprophylactic trial, conducted for six weeks and employing doses of 200 mg of either drug per day.

METHODS

Surveillance for Influenza

Active surveillance for acute viral respiratory-tract disease is maintained by the Infectious Diseases Unit at multiple sites in the Burlington area. Surveillance is based on throat swabs submitted for virus isolation and weekly summaries of patients' visits.

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Supported by a contract (N01 AI 02646) from the National Institute of Allergy and Infectious Diseases.

Eligibility for Participation in the Study

Volunteers were 18 to 45 years old; they were in good health and had no known cardiac, pulmonary, or neurologic illnesses. Women were excluded if they were pregnant or possibly pregnant. Volunteers with acute respiratory-tract illnesses during the two weeks before the study were also excluded. Volunteers were ineligible if they had received influenza vaccine during the past year or if they were taking medications that might interfere with the study.

Study Design

Participants were assigned to the rimantadine, amantadine, or placebo group under a double-blind, randomized allocation for the six-week duration of the study. Four hundred fifty participants were initially enrolled and sequentially assigned a number, previously allocated to one of three treatment groups from a table of random numbers.[13] On entry, volunteers gave a brief medical history and provided a serum specimen. They were then given a packet containing one week's supply of medication and instructed to take one tablet (100 mg) in the morning and one tablet in the evening for seven days. Every volunteer received a diary with which to record daily the presence or absence of respiratory and gastrointestinal symptoms or side effects. Each week, the volunteer returned the diary to the study center and received a new diary and another week's supply of medication. Volunteers were instructed to return at once if acute respiratory illness developed, and they were encouraged to do so even if the illness was mild. At each visit for respiratory illness, the volunteer was examined by a physician, and a throat swab and a serum sample were obtained. A serum sample was also obtained at the end of the sixth week, at the conclusion of the study.

Volunteers were instructed to return to the center at once if possible side effects developed, but they were asked to continue taking the medication if a respiratory illness occurred, since the cause of such illnesses could not be determined until laboratory tests were completed. To monitor compliance, a random 10 per cent sample of the study population was asked, unannounced, to provide a urine specimen to be tested for amantadine content. (The amantadine levels were kindly measured by D. C. Rakestraw of Endo Laboratories, Newark, Del.)

Before entry into the study, written informed consent was obtained from all volunteers according to guidelines established by the Department of Health and Human Services. These studies were approved by the University of Vermont Human Experimentation Committee, the University of Vermont Clinical Research Center Review Committee, and the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases.

Laboratory Procedures

For virus isolation, throat swabs were placed in veal-infusion broth supplemented with 0.5 per cent bovine serum albumin, and 0.2 ml was inoculated into tubes of each of the following cells: Madin–Darby canine kidney, primary rhesus-monkey kidney, continuous rhesus-monkey kidney, human diploid fibroblast, and human heteroploid (Flow Laboratories, McLean, Va., and M.A.

Bioproducts, Walkersville, Md.). Cultures were incubated for 14 days at 33°C, and influenza virus was detected by hemadsorption.[14] Isolates were identified by a direct fluorescent-antibody assay and by hemagglutination inhibition.[14] Serum specimens were tested for hemagglutination-inhibition antibodies to influenza A/Brazil/11/78 (subtype H1N1) and influenza A/Bangkok/1/79 (H3N2) and for complement-fixation antibodies to these viruses as well.[14] In addition, serum samples were tested for rises in both IgG and IgM antibodies to A/Bangkok and A/Brazil strains by an enzyme-linked immunosorbent assay.[15]

Definitions of Influenza-like Illness and Infection with Influenza A Viruses

Influenza-like illness was defined by the presence of cough or fever (a temperature above 37.7°C as measured orally), or both, and at least two of the following symptoms: sore throat, headache, and myalgia. Determination of whether a volunteer had influenza-like illness was made by a physician member of the study team at the time of examination, before the availability of laboratory data.

Laboratory evidence of influenza A infection was defined by any of the following findings: isolation of influenza A virus, a fourfold or greater rise of hemagglutination-inhibition or complement-fixation antibodies to A/Bangkok or A/Brazil, or a statistically significant rise in IgG or IgM antibodies to either of the above viruses, as detected by the enzyme-linked immunosorbent assay.

Analysis of Data

Differences in the rates of illness, infection, and side effects were analyzed by chi-square tests. The reduction in the rate of illness in drug recipients (the efficacy rate) was calculated as follows:

$$\frac{\text{rate of illness in placebo group} - \text{rate in amantadine or rimantadine group}}{\text{rate in placebo group}} \times 100.$$

Results

Detection of Influenza Activity in the Community

The pattern of isolation of influenza viruses from samples obtained through the surveillance system is shown in Figure 1. H3N2 isolates, similar to A/Bangkok/1/79, were detected through the week of January 17, 1981, at which time H1N1 viruses, similar to A/Brazil/11/78, were isolated. Because of the increasing number of influenza-virus isolates, along with increases in the rates of respiratory illnesses seen at the surveillance sites during early January (data not shown), the trial was begun during the week of January 10.

Characteristics of the Study Population

A total of 450 volunteers were enrolled into the study. Ten volunteers dropped out within 24 hours after enrollment but before taking any medication, primarily because of inability to comply with the study requirements. Of the remaining volunteers, 148 received placebo, 147 rimantadine, and 145 amantadine. The mean age of the volunteers (±S.E.M.) was 25.60±0.45 years. There were no significant differences in age, race, male:female ratios, or levels of preexisting serum hemagglutination-inhibition antibodies to A/Brazil or A/Bangkok among the study groups.

Influenza-like Illness in the Study Population

The rates of influenza-like illness among placebo, rimantadine, and amantadine recipients are shown in Table 1. Rates are presented only for volunteers who completed the entire six weeks of the study (see below for a discussion of the dropout rates). Significantly more placebo recipients (41 per cent) than rimantadine recipients (14 per cent) or amantadine recipients (9 per cent) had influenza-like illness ($P<0.001$ by chi-square analysis). The rates of illness in rimantadine and amantadine recipients were not significantly different. As compared with placebo, rimantadine reduced the rate of influenza-like illness by 65 per cent, whereas amantadine reduced the rate by 78 per cent.

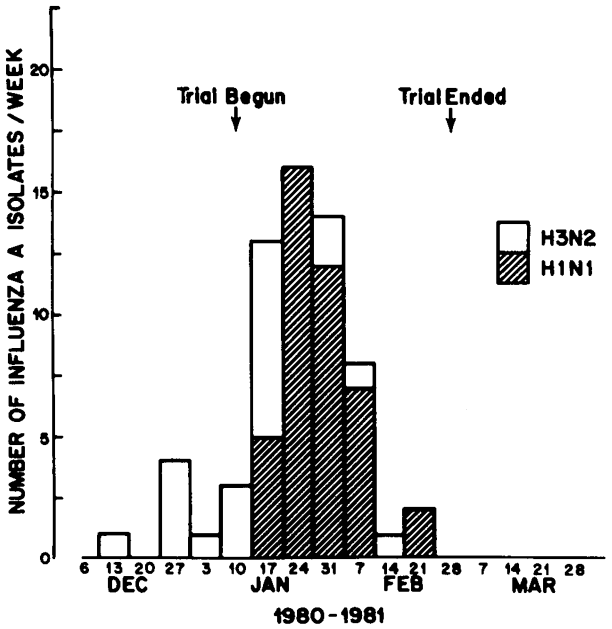

Figure 1. Influenza A Virus Isolates of H3N2 and H1N1 Subtypes, Obtained through the Respiratory-Virus Surveillance System at the University of Vermont, 1980–1981.

Arrows indicate the beginning and end of the trial of chemoprophylaxis.

Laboratory-Documented Influenza in the Study Population

The rates of influenza-like illness associated with laboratory-documented influenza A infection ("laboratory-documented influenza") are shown in Table 1. Rates of laboratory-documented influenza were significantly lower in both rimantadine and amantadine recipients than in placebo recipients ($P<0.001$). This high degree of efficacy was reflected in reductions of 85 per cent and 91 per cent in the rates of laboratory-documented influenza with rimantadine and amantadine, respectively, as compared with placebo. The efficacy rates for the prevention of laboratory-documented influenza were greater than those for the prevention of all influenza-like illnesses in both the amantadine and rimantadine groups (Table 1).

Of the 20 isolates of influenza A virus obtained from study participants, 18 were of the H1N1 subtype and two were of the H3N2 subtype. Among infections detected only by serology, 79 per cent were caused by H1N1 viruses and 21 per cent by H3N2 viruses. In

Table 1. Influenza-like Illness, Laboratory-Documented Influenza, and Infection with Influenza A Virus among Volunteers Receiving Placebo, Rimantadine, or Amantadine.

Treatment Group (No. of Subjects)	No. with Influenza-like Illness *	No. with Laboratory-Documented Influenza †	No. Infected with Influenza A Virus ‡
Placebo (132)	54 (41%)	27 (21%)	32 (24%)
Rimantadine (133)	19 (14%) §	4 (3%) §	11 (8%) §
Efficacy rate (%) ¶	*65*	*85*	*66*
Amantadine (113)	10 (9%) §	2 (2%) §	7 (6%) §
Efficacy rate (%) ¶	*78*	*91*	*74*

*Defined as a cough or an oral temperature of >37.7°C, or both, and at least two of the following: sore throat, headache, and myalgia. Figures in parentheses indicate percentages of subjects with indicated finding.

†Defined as influenza-like illness along with virus isolation or a rise in serum antibody to influenza A virus.

‡Defined as influenza A virus isolation or a rise in serum antibody to influenza A virus, irrespective of the presence of illness.

§P<0.001 as compared with placebo by chi-square analysis.

¶Efficacy rates are calculated by the expression:

$$\frac{\text{rate in placebo recipients} - \text{rate in rimantadine or amantadine recipients}}{\text{rate in placebo recipients}} \times 100.$$

addition, two isolates of parainfluenza virus (types 2 and 3) and two isolates of herpes simplex virus (type 1) were obtained.

Influenza A Infection in the Study Population

The rates of infection with influenza A viruses, irrespective of the subjects' clinical history, were significantly reduced by both rimantadine and amantadine (P<0.001) (Table 1). The efficacy rates for the prevention of infection by both rimantadine (66 per cent) and amantadine (74 per cent) were lower than those observed for the reduction of laboratory-documented influenza A (Table 1).

Compliance with the Medication Regimen

Urine specimens to be tested for drug content were requested from 38 subjects (approximately 10 per cent of those who completed the study). Because such selections were made independently of drug allocation, 21 of the samples came from placebo recipients, eight from rimantadine recipients, and nine from amantadine recipients. Eight of nine subjects who received amantadine (89 per cent) had detectable levels of amantadine in urine (52 to 438 μg per milliliter). Assays for rimantadine were not available.

Side Effects in the Study Population

In addition to the 10 volunteers who dropped out during the first day, 62 dropped out during the six weeks of the study (Table 2). Withdrawal rates were significantly higher in amantadine recipients (22 per cent) than in either placebo recipients (11 per cent; P<0.01) or rimantadine recipients (10 per cent; P<0.005). The excess withdrawal rate in the amantadine group can be accounted for largely by the development of side effects on the central nervous system in 13 per cent of the amantadine recipients, as compared with 6 per cent of the rimantadine recipients (P<0.05) and 4 per cent of the placebo recipients (P<0.01). The central-nervous-system side effects that were reported were primarily insomnia, "jitteriness," and difficulty in concentrating. Symptoms generally cleared within 48 hours after therapy was stopped. One amantadine recipient, who had a previously unreported history of syncopal episodes, had "dizziness" followed by a syncopal attack and withdrew from the study. This episode was included as a central-nervous-system side effect associated with the drug.

The frequency of other (non-central-nervous-system) side effects was not significantly different among the study groups. These included gastrointestinal distress in five subjects and a rash in one. A variety of reasons for withdrawal, unrelated to side effects, were given by 15 subjects. These reasons were primarily related to difficulty in complying with the schedule of visits. Two volunteers (one placebo recipient and one amantadine recipient) stopped taking the medication when respiratory illness developed; these dropouts are included as withdrawals unrelated to side effects. Reasons for withdrawal could not be ascertained in seven volunteers.

Discussion

An outbreak of influenza A in Burlington during 1981 enabled us to conduct a large-scale, direct comparison of the prophylactic efficacies of rimantadine and amantadine. Because the trial was initiated early in the outbreak, a high attack rate was observed in the study population, so that the effectiveness of rimantadine and that of amantadine could be purposefully compared.

At the doses employed in the trial (200 mg per day for six weeks), both rimantadine and amantadine were highly effective in preventing influenza-like illness, with reductions in the rates of illness of 65 per cent and 78 per cent, respectively, as compared with placebo. The effects were even more striking when reductions in the rates of laboratory-documented influenza were analyzed, with efficacy rates of 85 per cent for rimantadine and 91 per cent for amantadine. The prophylactic efficacy of amantadine in this study was similar to the

Table 2. Withdrawal Rates among Recipients of Placebo, Rimantadine, or Amantadine.

Treatment Group (No. of Subjects)	Withdrawals	Reasons for Withdrawal			
		CNS side effects *	Non-CNS side effects	Unrelated to side effects	Unknown
		no. of subjects (%)			
Placebo (148)	16 (11)	6 (4)	1 (0.7)	8 (5)	1 (0.7)
Rimantadine (147)	14 (10)	9 (6)	1 (0.7)	4 (3)	0 (0)
Amantadine (145)	32 (22) †	19 (13) ‡	4 (3)	3 (2)	6 (4)

*Primarily insomnia, "jitteriness," and difficulty in concentrating. CNS denotes central nervous system.

†P<0.01 as compared with the placebo, and P<0.005 as compared with rimantadine, by chi-square analysis.

‡P<0.01 as compared with placebo and P<0.05 as compared with rimantadine.

efficacy reported in most other field trials.[4-6,8,16] Data on the prophylactic efficacy of rimantadine in field trials have been restricted to reports from the Soviet Union, where efficacy rates of 70 per cent or higher have been claimed, largely on the basis of uncontrolled observations.[12] Rimantadine had been reported to be effective in the prophylaxis of experimentally induced influenza in volunteers[17] and was shown to have a small therapeutic effect in uncomplicated influenza in a recent study.[18]

Studies in which the prophylactic efficacy rates of rimantadine and amantadine could be directly compared have not been previously reported. Two recent trials in which rimantadine and amantadine were administered prophylactically to college students were initiated late in an influenza outbreak, and definitive conclusions about the relative efficacies of the two compounds could not be reached.[19,20] In our study, both amantadine and rimantadine were highly effective, and there were no significant differences between the rates of illness or infection in the two drug-treated groups.

However, significant differences in the rates of side effects in rimantadine and amantadine recipients were observed. Rimantadine was associated with side effects no more frequently than placebo. In contrast, amantadine recipients had a higher rate of side effects than either placebo or rimantadine recipients. This higher rate was largely attributable to central-nervous-system symptoms that caused enough discomfort to result in withdrawal from the study. The excess rate of central-nervous-system side effects in amantadine recipients was 9 per cent as compared with the rate in placebo recipients and 7 per cent as compared with the rate in rimantadine recipients (Table 2). Such side effects reversed rapidly when the medications were discontinued.

The rates of side effects reported in previous studies with amantadine have varied widely, but several studies have reported rates similar to those observed in this study.[5,6,8,16,21-23] In the two trials mentioned above in which amantadine and rimantadine were both administered, low rates of side effects in recipients of each drug were reported.[19,20] The reasons for the differences from the rates in our study are not clear but may be related to the nature of the study populations or to differences in rates of compliance. Although limited data are available, compliance with the medication regimens in our study appeared to be excellent, on the basis of the efficacy rates and the urinary levels of amantadine in randomly selected subjects. It is of interest that a recent study examining the therapeutic efficacy of these compounds reported a higher rate of side effects for amantadine than for placebo or rimantadine.[18]

As noted above, both amantadine and rimantadine appeared to be less effective in preventing infection with influenza A virus than in preventing infection-associated illness. This phenomenon, previously noted in trials with amantadine,[8,16,23] may be a potentially desirable feature of prophylaxis, since subclinical infection could confer immunity against reinfection.

This study indicates that chemoprophylaxis with rimantadine or amantadine, instituted early in an outbreak of influenza A, can result in a marked decrease in rates of influenza. At the doses employed here, rimantadine and amantadine appeared to be equally effective, but rimantadine was associated with significantly fewer side effects. On the basis of this study, rimantadine appears to be the drug of choice for the chemoprophylaxis of influenza A. It should be pointed out that our studies were conducted in healthy young volunteers. Whether similar findings, including the low rate of side effects, would be encountered in the elderly or other high-risk persons requires additional study.

We are indebted to Dr. John La Montagne and Mrs. Patricia Stewart of the National Institute of Allergy and Infectious Diseases for randomization of the medications.

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HISTOLOGICAL OSTEOMALACIA DUE TO DIETARY CALCIUM DEFICIENCY IN CHILDREN

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Abstract We performed a histomorphometric study of trabecular-bone formation and resorption in undecalcified sections of iliac crest from three children presenting with clinical, radiologic, and biochemical evidence of rickets associated with dietary calcium deficiency. All three children had severe osteomalacia documented by hyperosteoidosis and reduced static and dynamic indicators of bone mineralization. There was a reduction of the calcified bone volume associated with a decreased bone formation rate and features of increased bone resorption. Correction of dietary calcium intake in two of the patients led to normal serum and urinary calcium levels and reduced alkaline phosphatase levels. After calcium therapy, the calcified bone volume was normal and indicators of bone mineralization returned to normal.

We conclude that low calcium intake in children may be associated with a histologic picture of severe osteomalacia. Our finding that adequate amounts of calcium rapidly improved bone mineralization demonstrates that calcium deficiency can cause osteomalacia in children. (N Engl J Med. 1982; 307:584-8.)

ALTHOUGH an adequate intake of calcium is required for optimal growth and mineralization of the skeleton, the effects of calcium deficiency on bone metabolism remain uncertain. In many species, a low-calcium diet leads to bone loss and osteopenia.[1-4] In adults, dietary calcium deficiency is associated with increased secretion of parathyroid hormone and increased bone resorption — a process that is thought to contribute to the development of senile osteoporosis.[1] In children, calcium deficiency has occasionally been found to cause rickets.[5,6] Although suspected,[7] histologically confirmed osteomalacia due to calcium deficiency itself has not been described in human beings. Recently, a syndrome characterized by radiologic, clinical, and biochemical features of rickets with normal serum concentrations of 25-hydroxyvitamin D (25-(OH)D) has been described in a rural black population.[8,9] Calcium deficiency was thought to be the most likely cause of the disorder, since the rickets were healed by a calcium-supplemented diet without vitamin D therapy.[10] Although these children had clinical bone deformities and radiologic evidence of rickets on admission, the actual bone pathology remained unknown. Our report deals with the quantitative histologic evaluation of bone pathology in this condition.

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Supported by the Shriners of North America, the South African Medical Research Council, and the Atomic Energy Board.

PATIENTS

Three black children, one girl four years old and two boys six and 13 years old, were studied. They came from rural farming communities located in the southeastern Transvaal, South Africa, where they had lived all their lives. Previous reports have indicated that the pediatric population in this area has a high prevalence of hypocalcemia.[9] The diet of these three children consisted mainly of maize with vegetables and occasional meat, but milk and milk products were not consumed. A dietary survey of clinically affected children from the same area revealed a very low dietary calcium intake of 125 mg per day but a normal phosphorus intake.[10]

The three patients presented with clinical syndromes similar to those previously described.[8,9] On admission, the two boys had weights and heights below the third percentile for their ages, as well as mild bone deformities of the lower limbs. Radiologic examination of the three patients revealed active rickets at the wrist or knees, and the long bones appeared osteopenic. No overt evidence of undernutrition other than growth retardation was noticed. On admission, the children received a normal ward diet, with no vitamin D supplements, containing approximately 1000 mg of calcium and 800 mg of phosphorus per day. In two of the three patients, calcium intake was further increased by daily administration of 1000 mg of calcium (calcium Sandoz forte) for five to eight months, during which radiologic, biochemical, and histologic studies were performed.

METHODS

Blood and urine samples were taken in the morning under fasting conditions. Serum and urinary concentrations of calcium and magnesium were measured with a Varian atomic absorption spectrophotometer. Serum and urinary phosphorus and creatinine and serum alkaline phosphatase were measured on a Technicon AutoAnalyzer II. The renal threshold for phosphate reabsorption was calculated according to the nomogram of Walton and Bijvoet.[11] Serum 25-(OH)D concentrations were measured by the competitive protein-binding method of Haddad and Chyu.[12] Reference biochemical values were obtained from normal black children.

A transiliac iliac-crest bone biopsy was performed with a 6-mm Bordier trephine before and after 140 or 224 days of calcium supplementation. Two of the three patients underwent a course of double