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Short communication

Long-term stability of the anti-influenza A compounds—amantadine and rimantadine

Christoph Scholtissek, Robert G. Webster *

Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale, P.O. Box 318, Memphis, TN 38101, USA

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Abstract

Amantadine and rimantadine hydrochloride were tested for stability after storage at different temperatures and under different conditions for extended periods of time. Both compounds were quite stable after storage for at least 25 years at ambient temperature; they both retained full antiviral activity after long-term storage or after boiling and holding at 65–85°C for several days. Thus, amantadine and rimantadine could be synthesized in large quantities and stored for at least one generation without loss of activity in preparation for the next influenza A pandemic in humans.

Keywords: Anti-influenza compounds; Amantadine; Rimantadine

Amantadine is a derivative of adamantane that efficiently inhibits replication of influenza A viruses (Davies et al., 1964). This drug inhibits replication by interfering with the ion channel activity of the matrix (M)-2 protein (Sugrue and Hay, 1991; Pinto et al., 1992; Schroeder et al., 1994) and/or with the pH-dependent conformational change of the hemagglutinin (HA), which is necessary for fusion of the endosomal and viral membranes (Daniels et al., 1985; Steinhauer et al., 1991). Because influenza viruses (like other RNA viruses) have a relatively error-prone polymerase and no repair system, drug-resistant variants can be obtained easily by passing the virus in the presence of amantadine (Cochran et al., 1965; Appleyard, 1977). The strains obtained by passing the virus in the presence of relatively low concentrations of amantadine (≈ 1 μg/ml) contained amino acid replacements at one of four specific

* Corresponding author. Tel.: +1 901 4953400; fax: +1 901 5232622; e-mail: robert.webster@stjude.org

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Table 1
Inhibition of plaque formation by amantadine and rimantadine hydrochloride

Amantadine or rimantadine hydrochloride in overlay (μg/ml)	Number of plaque-forming units ($\log_{10}$) after treatment with the following amantadine or rimantadine hydrochloride preparations						
	1	2	3	4	5	6	7
0	8.04/8.24						
0.04	7.60	7.92	8.00	7.87	NT	NT	NT
0.05	8.14	8.00	8.09	NT	5.30[a]	5.14[a]	5.00[a]
0.08	7.84	7.92	7.50	7.97	NT	NT	NT
0.10	8.00[b]	8.00[b]	7.20[b]	NT	0[c]	0[c]	0[c]
0.12	7.60[d]	7.40[d]	7.00[d]	7.37[d]	NT	NT	NT
0.16	7.00[a]	7.00[a]	6.20[a]	6.76[a]	0[c]	0[c]	0[c]
0.20	0[c]	0[c]	0[c]	0[c]	NT	NT	NT
0.40	0	0	0	0	NT	NT	NT

Preparations: 1, newly purchased amantadine hydrochloride (Sigma); 2, amantadine stored at room temperature ($\approx$20°C) at SJCRH for at least 25 years; 3, amantadine stored at 4°C in Giessen for at least 20 years; 4, amantadine hydrochloride (Giessen) made up to 1 mg/ml in PBS, heated at 100°C for 60 min and held at 65°C for 5 days; 5, newly purchased rimantadine hydrochloride (Forest Pharmaceuticals); 6, rimantadine stored at room temperature ($\approx$20°C) at SJCRH for at least 25 years; and 7, rimantadine heated at 100°C for 120 min and held at 80°C for 4 days.
A/Singapore/1/57 (H2N2) was diluted to produce approximately 8.0 ($\log_{10}$) plaques in MDCK cells and treated with amantadine and rimantadine hydrochloride in the overlay at the above concentrations.
NT, not tested.
[a]Very small plaques ($\approx$95% reduction) in all drug preparations tested.
[b]Plaques reduced in size by $\approx$50% or more in all drug preparations tested.
[c]A few minute plaques in each drug preparation tested.
[d]Plaques reduced in size by $\approx$75% or more in all drug preparations tested.

sites in the membrane-spanning region of the M2 ion channel (Hay et al., 1985).

In preparation for the next human influenza A pandemic, strategic plans have been developed in many countries on steps that can be taken to prepare for this event. One possibility considered was the preparation and storage of the two licensed anti-influenza A compounds—amantadine and rimantadine. At a symposium entitled Pandemic Influenza: Confronting a Reemerging Threat, held in Bethesda, MD in December 1995, questions were raised about the stability of amantadine and rimantadine. Currently these two drugs have a short FDA approval time of only 2 years.

To investigate the stability of these two compounds, studies were done on drug preparations available in two different laboratories. One batch of amantadine hydrochloride and one of rimantadine hydrochloride (Dupont, Wilmington, DE) were kept for at least 25 years at room temperature ($\approx$20°C) at St. Jude Children's Research Hospital (SJCRH), Memphis, TN, and another batch of amantadine hydrochloride (Sigma, St. Louis, MO) was kept at 4°C for at least 20 years at the Institute of Virology in Giessen, Germany. These drugs were compared with freshly purchased amantadine (Sigma, St. Louis, MO) and rimantadine hydrochloride (Forest Pharmaceuticals, USA). Stock solutions of 1 mg/ml in phosphate buffered saline (PBS) were prepared and 1 ml of the Giessen amantadine stock solution was boiled for 1 h and kept for 5 days at 65°C, and 1 ml of the Memphis rimantadine stock solution was boiled for 2 h and kept for 4 days at 80°C.

Madin Darby Canine Kidney (MDCK) cells were used throughout the study and were infected with A/Singapore/1/57 (H2N2) at appropriate dilutions starting with approximately 10^8 plaque-forming units/ml and overlaid with agar in Eagle's minimal essential medium containing 4% bovine serum albumin, 0.5 μg/ml trypsin, and the drugs

at various concentrations as given in Table 1. For each dilution one monolayer was used. Each experiment was conducted at least twice. The maximal difference in two independent experiments were 0.3 $\log_{10}$ units.

Each of the preparations of amantadine hydrochloride inhibited plaque formation by A/Singapore/1/57 (H2N2) influenza virus and there were no detectable differences between the preparations (Table 1). It is noteworthy that each of the preparations caused plaque size reduction at the same concentrations and that heating to 100°C and storage at 65°C for 5 days also failed to decrease the biological activity of amantadine hydrochloride. Comparable results were obtained with rimantadine. At the lowest dose tested (0.05 μg/ml), only a few micro plaques were observed with all three samples. Higher doses completely inhibited plaque formation by A/Singapore/1/57 (Table 1).

These results indicate that both amantadine and rimantadine are extraordinarily stable, and would be ideal for long-term storage for use in the initial stages of a new influenza A pandemic. According to our estimates, it would be difficult even for our grandchildren to determine the ex vivo decay time of these compounds. After a new influenza A pandemic has appeared, and while a vaccine is under development, amantadine and rimantadine are the only FDA-approved drugs for treatment and prevention.

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