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Isolation of Amantadine-Resistant Influenza A Viruses (H3N2) from Patients following Administration of Amantadine in Japan

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In Japan, the use of amantadine for treatment of influenza A virus infection was not accepted until November 1998, although it was widely used for treatment of Parkinsonism. Since then, we have monitored the emergence of amantadine-resistant viruses and isolated two viruses from patients on long-term treatment with amantadine.

Amantadine and rimantadine have been used for the prevention and treatment of influenza A virus infection (4, 10, 16). They block the proton flow through the M2 ion channel and prevent the release of viral ribonucleoprotein complex into the cytoplasm of infected cells (6, 10). Patients who received amantadine or rimantadine for the treatment of influenza A virus infection were sometimes found to produce viruses resistant to these drugs (3, 5, 7, 9, 11, 12). It has been reported that a single change in one of five amino acid residues in the transmembrane portion of the M2 protein (residues 26, 27, 30, 31, and 34) results in complete resistance to amantadine and rimantadine (10).

In Japan, the use of amantadine for the treatment or prevention of influenza A virus infection was not accepted until November 1998, although it had been widely used to treat Parkinsonism, neuropsychiatric disorders, and apathy due to cerebral infarction (13, 15). Therefore, the ability of amantadine to prevent influenza A virus infection in patients who received long-term treatment with the drug was not studied. Since November 1998, we have monitored the emergence of viruses resistant to amantadine in a hospital in Kyushu in order to examine emergence of the amantadine-resistant viruses among patients on long-term amantadine administration. We isolated two amantadine-resistant viruses (H3N2) during the 1998–1999 influenza season.

Study subjects were 10 patients in the 1998–1999 influenza season and 16 patients in the 1999–2000 influenza season who had Parkinsonism and postcerebral infarction syndrome (age [mean ± standard deviation], 78.4 ± 5.6 years) and took amantadine (50, 100, or 150 mg/day) orally for more than 6 months before and during the influenza season. Study controls were 20 patients who in the 1998–1999 influenza season had neuropsychiatric disorders and apathy due to cerebral infarction but did not take amantadine (average age, 78.2 ± 4.8 years). No patients were vaccinated in the hospital. Subjects were promptly seen whenever influenza-like illness occurred (fever of ≥37.8°C plus at least two of the following signs and symptoms: headaches, malaise, myalgia, sore throat, pain on coughing, and anorexia) in the influenza season. Influenza virus isolation from a nasopharyngeal swab was performed on MDCK cells, and subtypes of isolated viruses were identified using standard sera against A/Beijing/262/95 (H1N1), A/Sydney/05/97 (H3N2), B/Harbin/07/94, and B/Beijing/243/97. Titers of hemagglutination inhibition (HI) against these viruses in their paired sera were also measured.

In the 1998–1999 influenza season, five patients (50%) taking amantadine and three patients (15%) not taking amantadine were confirmed as influenza A virus (H3N2) infected by a >4-fold increase in HI titers of paired sera. Three influenza A viruses (H3N2), AD(+)1, AD(+)2, and AD(+)3, were isolated from the patients taking amantadine, and three viruses, AD(−)1, AD(−)2, and AD(−)3, were isolated from those not taking the drug. In the 1999-2000 influenza season, none of the 16 patients taking amantadine had a confirmed influenza A virus infection.

The M gene of the isolated viruses was cloned using an RNA PCR kit (Takara). Briefly, 10 ng of RNA extracted from the isolated viruses was reverse transcribed using primer TT168 (26-ATGAGCCTTCTAACCGAGGTCG-47), followed by 30 cycles of PCR with the primer pair TT168 and TT169 (916-A CTCCTTCCGTAGAAGGCCC-897) at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The amplified fragments were cloned into pT7Blue-T vector (Novagen), and three independent clones of each virus were sequenced by an ABI Prism 310 using the BigDye terminator cycle sequencing ready reaction kit (PE Applied Biosystems).

Two viruses from the patients taking amantadine carried amantadine-resistant amino acid mutations in M2 protein: Ala30 → Val [AD(+)1] and Ser31 → Asn [AD(+)2]. These mutations have been identified as amantadine-resistant markers of M2 (6, 8, 10). However, AD(+)3 and three viruses from the patients not taking amantadine carried amantadine-sensitive sequences. AD(+)1 and AD(+)2 made plaques in the presence of 25 μg of amantadine per ml, while others did not make plaques in 2 μg of amantadine per ml (data not shown).

Antiviral mechanisms of amantadine and amino acid sequences of the amantadine-resistant M2 protein are well stud-

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ied (6, 10). Apparent transmission of amantadine-resistant viruses to close, susceptible contacts in families and other semiclosed settings has been described (7). Amantadine-resistant viruses have been reported to emerge in the immunocompromised host (5) or the family members of those who were treated with amantadine (2).

This is the first report of isolation of amantadine-resistant viruses from patients receiving long-term amantadine treatment for Parkinsonism, neuropsychiatric disorders, and apathy due to cerebral infarction. It was reported previously that postexposure prophylaxis with amantadine sometimes failed and resulted in the emergence of the resistant viruses (8). AD(+)1 and AD(+)2 were isolated from the patients taking 150 mg of amantadine/day, and AD(+)3 was isolated from those taking 100 mg/day. The plasma amantadine concentration of the patients was measured three times by the method of Zhou and Krull (18) during the period and was always higher than 1 μg/ml. The question arises as to how the amantadine-resistant viruses could appear in the presence of more than 1 μg of amantadine per ml of serum, because the concentration was enough to inhibit influenza virus replication (14). Although the patients were elderly (average age, 78.4 ± 5.6 years), we did not find any evidence of apparent immunodeficiency in them. We also did not find any difference in the course of infection among them. Our data indicated that long-term amantadine administration did not always prevent influenza A virus infection. All influenza viruses may not become resistant under the evolutionary pressure of amantadine, because the amantadine-sensitive virus AD(+)3 was isolated from the patients taking the drug.

Our results indicate that the amantadine-resistant viruses may naturally circulate and emerge from patients receiving long-term treatment with amantadine. These viruses seemed genetically stable because they could be transmitted through six successive generations of exposed chickens (1). However, the surveillance done by other groups indicated that the incidence of emergence of amantadine and rimantadine resistance in field isolates was low (2, 17, 19). Our 2-year surveillance in the same hospital agreed with their results because we had no evidence of expansion of the amantadine-resistant virus population in the second influenza season.

Nucleotide sequence accession numbers. The sequences reported in this paper were deposited to DDBJ, EMBL, and GenBank under the accession numbers AB036067 and AB036068.

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