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Dual Mutations in the HA1 Peptide of Amantadine-Resistant Influenza Viruses at Positions 193 and 225

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An increase in the proportion of amantadine-resistant influenza A viruses began in 2002-2003 in China and Hong Kong, and in 2005-2006, an extremely high frequency of these viruses was observed in the United States. Similar tendencies have been reported in Australia, New Zealand, Southeast Asia, Macau and Cambodia (1). In many cases, amantadine-resistant viruses show an amino-acid substitution in the M2 protein at position 31 (S31N). Saito et al. reported that viruses with mutations not only in the M2 gene but also in the HA1 gene, S193F and D225N, were isolated in 2005 (2). In our previous report, we showed a high frequency (72.2%, 13 out of 18) of amantadine-resistant influenza AH3 viruses in the 2005-2006 season in Nara Prefecture, Japan.
Here, we report the results of HAI (hemagglutinin) gene analysis of the same 18 isolated viruses.

In order to search for mutations in the HAI gene, we amplified DNA fragments (prospective size, 1,073 bp) by the reverse transcription-polymerase chain reaction method using specific primers (H3F1 and H3R1) reported by Besselaar et al. (4). Amino acid substitutions were confirmed by partial nucleotide sequencing. The obtained genetic sequences of the HAI gene were compared with drug-sensitive virus sequences and analyzed in GenBank using a BLAST search. The results showed that all of the 13 drug-resistant isolates contained an amino-acid substitution: serine (S) to phenylalanine (F) (AGC→TTC) at position 193. In addition, 11 of the 13 resistant isolates (84.6%) showed another mutation at position 225: asparaginic acid (D) to asparagine (N) (GAT→AAT). A BLAST search revealed that the 11 strains shared high homology with strains A/New York/5/2006, A/New York/4/2006 and A/Malaysia/778/2005 (GenBank accession no. CY013233, CY012793 and DQ849023, respectively). These strains had not only a point mutation in the M2 gene, but also dual mutations in the HAI gene. In contrast, the 5 drug-sensitive isolates showed no such amino-acid substitutions.

Saito et al. suggested that viruses isolated in Nagasaki, Japan and Vietnam in 2005 had dual mutations in the HAI gene as well as a single mutation in the M2 gene and belonged to the same genetic lineage (2). HA protein plays an important role in virus infection, and the dual mutations in the HAI gene may have caused the significant increase in the frequency of the drug-resistant virus in the 2005-2006 season. The present study proved that the existence of amantadine-resistant viruses with a single mutation in the HAI gene at position 193. These results suggest that the mutation at position 193 may contribute independently to the infectivity of influenza viruses. However, the level of the contribution to infectivity of each point mutation and the implications of dual mutations remain unknown.

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