

AVIANFLU

Isolation of drug-resistant H5N1 virus

The persistence of H5N1 avian influenza viruses in many Asian countries and their ability to cause fatal infections in humans have raised serious concerns about a global flu pandemic¹. Here we report the isolation of an H5N1 virus from a Vietnamese girl that is resistant to the drug oseltamivir², which is an inhibitor of the viral enzyme neuraminidase and is currently used for protection against and treatment of influenza. Further investigation is necessary to determine the prevalence of oseltamivir-resistant H5N1 viruses among patients treated with this drug.

An H5N1 influenza virus, A/Hanoi/30408/2005, was isolated on 27 February 2005 from a 14-year-old Vietnamese girl (patient 1) who had received a prophylactic dose (75 mg once a day) of oseltamivir from 24 to 27 February and was given a therapeutic dose (75 mg twice daily) for 7 days starting on 28 February. No virus was isolated from specimens after the administration of increased doses of oseltamivir. The patient recovered and was discharged from hospital on 14 March 2005.

Direct sequencing after amplification by polymerase chain reaction of the virus isolated from a specimen collected on 27 February indicated that some of the virus population had a histidine-to-tyrosine substitution at position 274 (represented as H274Y) in its neuraminidase protein, a mutation that confers resistance to oseltamivir^{3–5}. We therefore tested the sensitivity of the virus to oseltamivir carboxylate⁶ (the active form of the drug) and

found that the dose required for 50% inhibition of neuraminidase activity (IC_{50}) in the isolate was 90 nM, which exceeds the IC_{50} for oseltamivir-sensitive viruses (0.1–10 nM)⁷. We then plaque-purified the virus.

Ten viral clones, randomly picked from the resultant plaques, were classified into three groups according to their response to oseltamivir (see supplementary information): six were highly resistant to the drug ($IC_{50} > 763$ nM), three were slightly resistant (IC_{50} values between 7.1 and 12.5 nM) and one was highly sensitive ($IC_{50} = 0.6$ nM). The highly resistant viruses had tyrosine at position 274 in their neuraminidase, whereas those showing only slight resistance had serine at position 294.

Patient 1 had not had any known direct contact with poultry, but had cared for her 21-year-old brother (patient 2) while he had a documented H5N1 virus infection (for details of the disease course and treatment in these patients, see supplementary information). We found that the neuraminidase gene of the brother's virus was identical to clone 7 of the girl's virus (see supplementary information). Also, the haemagglutinin gene of the brother's virus was identical to clones 2 and 9 of the girl's virus, apart from a nucleotide change at position 271. The timing of infection in these two patients, together with the lack of known interaction of the girl with poultry, raises the possibility that the virus could have been transmitted from brother to sister.

We assessed the growth of a highly oseltamivir-resistant clone (H274Y, clone 9) and of an oseltamivir-sensitive clone (H274, clone 7) in ferrets⁸. Viral titres were higher in animals infected with the oseltamivir-sensitive virus (Fig. 1a; $P = 0.000099$; two-way repeated measures ANOVA). Oseltamivir treatment reduced viral titres in animals infected with the drug-sensitive virus (Fig. 1b; $P = 0.048$, Student's *t*-test), but not in animals infected with the resistant virus (Fig. 1b; $P = 0.23$, Student's *t*-test). However, all of the viral clones, including those highly resistant to oseltamivir, were sensitive to zanamivir^{9,10} (IC_{50} , 0.5–3.1 nM), another neuraminidase inhibitor. In ferrets, we found that zanamivir treatment reduced viral titres in animals infected with virus that was oseltamivir-sensitive (Fig. 1b; $P = 0.0000019$, Student's *t*-test) or oseltamivir-resistant (Fig. 1b; $P = 0.018$, Student's *t*-test).

We investigated how the viruses

bound *in vitro* to different configurations of sialyl glycopolymers, similar to those on the host's cell-surface receptor¹¹. We compared binding by two of the viral clones (clones 7 and 9) with binding by an avian flu virus (A/duck/Mongolia/301/2001) and another human flu virus (A/Kawasaki/1/2001). We found that both H5N1 clones bound to α -2,3-linked polymer and (less efficiently) to α -2,6-linked polymer (Fig. 1c, clone 7; clone 9, not shown). The A/duck/Mongolia/301/2001 virus also bound α -2,3-linked polymer but did not bind α -2,6-linked polymer at all; the A/Kawasaki/1/2001 virus bound strongly to α -2,6-linked polymer but only weakly to α -2,3-linked polymer (results not shown). The broader binding properties of our H5N1 viral clones may reflect a degree of adaptation in human hosts.

Although our findings are based on a virus from only a single patient, they raise the possibility that it might be useful to stockpile zanamivir as well as oseltamivir in the event of an H5N1 influenza pandemic. They also highlight the importance of monitoring the emergence of drug resistance in H5N1 isolates from patients treated with neuraminidase inhibitors.

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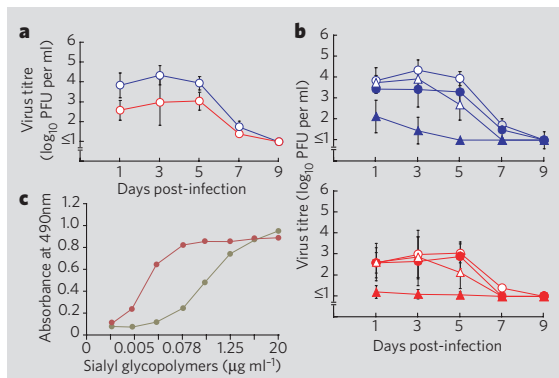


Figure 1 | Drug and binding sensitivity of H5N1 viral clones isolated from a human patient. **a, b**, Viral titres from ferrets (5 animals per group) infected with H5N1 viruses. PFU, plaque-forming units. **a**, Comparison of titres from oseltamivir-sensitive (blue) and oseltamivir-resistant (red) viruses in mock-treated animals (error bars, s.d.). **b**, Comparison of titres in animals treated with oseltamivir or zanamivir (error bars, s.d.). Top, oseltamivir-sensitive virus; bottom, oseltamivir-resistant virus: open symbols, mock-treated; filled circles, oseltamivir-treated; filled triangles, zanamivir-treated. **c**, Receptor specificity of viral clone 7 from patient 1, showing binding activity to sialyl glycopolymers containing either α -2,3-linked (red) or α -2,6-linked (brown) sialic acids. The maximum standard deviation of the data points shown was less than 0.09. For details of methods, see supplementary information.

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