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Structural basis for oseltamivir resistance of influenza viruses☆

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ABSTRACT

Oseltamivir, one of the two anti-neuraminidase drugs, is currently the most widely used drug against influenza. Resistance to the drug has occurred infrequently among different viruses in response to drug treatment, including A H5N1 viruses, but most notably has emerged among recently circulating A H1N1 viruses and has spread throughout the population in the absence of drug use. Crystal structures of enzyme–drug complexes, together with enzymatic properties, of mutants of H5N1 neuraminidase have provided explanations for high level oseltamivir resistance due to the common H275Y mutation, with retention of zanamivir susceptibility, and intermediate level resistance due to the N295S mutation. Complementation of enhanced NA activity due to a D344N mutation by the H275Y mutation suggests an explanation for the recent emergence and predominance of oseltamivir-resistant influenza A H1N1 viruses.

Determination of the 3D X-ray crystallographic structure of the influenza virus neuraminidase (NA) [1] provided the basis for one of the first examples of structure-based drug design [2] and the successful development of the licensed anti-influenza drugs zanamivir [2] and oseltamivir [3]. They differ in both the core ring structure and active moiety and target different parts of the enzyme catalytic site, providing the basis for potentially complementary resistance profiles. In addition to their role in therapy of seasonal influenza, these drugs are of particular importance as a first line defence against a pandemic, possibly caused by influenza A H5N1. Due to its oral bioavailability and easier administration, oseltamvir has been more widely used and is the principal component of drug stockpiles for use in the event of an emerging pandemic.

Initial studies in cell culture emphasized that resistance to the anti-NA drugs emerged much less readily than against the M2 channel inhibitors, amantadine and rimantadine. Furthermore, mutations were in general initially selected in HA, which reduced receptor binding affinity and consequently the requirement for active NA, prior to selection of mutations in NA which directly reduced inhibition of enzyme activity [4]. Resistance emerging *in vivo* has been principally associated with a few characteristic mutations in NA.

The frequency of resistance emergence in early clinical trials was also low, less than 1% for zanamivir and for oseltamivir approx. 0.3% in adults and somewhat higher, 4%, in children [5]. However, more recent studies in Japan [6] and the UK [5,7] have indicated that resistant viruses emerge more frequently in children treated with oseltamivir, with approx. 18% of children infected with influenza A H3N2 and 16–27% of those infected with A H1N1 shedding resistant virus. Of particular concern has been the emergence of oseltamivir-resistant viruses during treatment of A H5N1-infected patients, for example in one study 2 of 8 patients (25%) shed resistant virus, which was associated with fatal outcome [8]. The initial optimism has finally been dispelled by the recent emergence of oseltamivir-resistant A H1N1 viruses, in the absence of drug use, and their spread to become the predominant epidemic A H1N1 viruses [9,10].

The principal mutations responsible for conferring clinical resistance (*in vivo*) exhibit type and subtype-specific differences. In particular, those responsible for oseltamivir resistance of A H3N2 viruses have been R292K and to a lesser extent E119V, whereas for N1-containing viruses, H1N1 or H5N1, the most usual mutation conferring high resistance is H275Y (H274Y in N2 numbering) [11,12]. An N294S mutation in N2 (or N295S in N1 numbering) has been responsible for partial resistance to oseltamivir of H3N2 and H5N1 viruses [6,13]. For type B viruses, resistance to oseltamivir has been due to mutation of residue 198, D198N. The only reported instance of clinical resistance to zanamivir has been due to a R152K mutation in influenza B, although the principal mutations selected in cell culture have been in residue 119, E119G/A/D [11,12]. Furthermore, whereas the R152K mutation conferred cross-resistance to

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both zanamivir and oseltamivir, the oseltamivir resistance mutations in general cause substantially less resistance to zanamivir.

To understand the molecular basis of differences in resistance to the different drugs as well as the basis for subtype-specific differences, X-ray crystallographic structures were determined for resistant mutants in complex with drug. Such information for N1 may also help to understand the implications of the emergence of resistance in current H1N1 viruses for other N1-containing viruses, especially H5N1. Although the structures determined for N2, N9 and B NAs indicated they possessed a similar relatively inflexible catalytic site, more recent studies have shown that the different subtypes fall into 2 structurally distinct phylogenetic groups, group 1 comprising N1, N4, N5 and N8 and group 2 including N2, N3, N6, N7 and N9 [14]. The most notable difference was an additional cavity (150 cavity) adjacent to the active site of group 1 NAs. Although the functional significance of this difference is unclear, this feature provides an additional target for developing other anti-NA drugs. Other group-specific differences in the active sites have provided explanations for certain subtype-specific differences in resistance determinants. Thus comparison of the structures of N1 (of H5N1, group 1) and N9 (of H1N9, group 2) showed that, in relation to the R292K resistance mutation in N2 of H3N2 viruses, the loss of a H-bond from Arg 292 to the carboxylate of oseltamivir is complemented by a H-bond from Tyr 344 of N1 (of H5N1), helping to abrogate loss of drug susceptibility. On the other hand, the failure of the H275Y substitution to render N2 resistant to oseltamivir appears to be due to the greater space available to accommodate the larger Tyr residue, by a combination of the conformation of the 270-loop and the size of residue 252, Thr in N2 but Tyr in N1 [14].

The structure of the H275Y mutant of the NA of A/Vietnam/1203/2004 (H5N1) in complex with oseltamivir demonstrated how the bulkier Tyr residue alters the orientation of the key Glu 277 [15]. On binding oseltamivir, the conformation of the Glu 277 side chain of the wt enzyme is altered such that

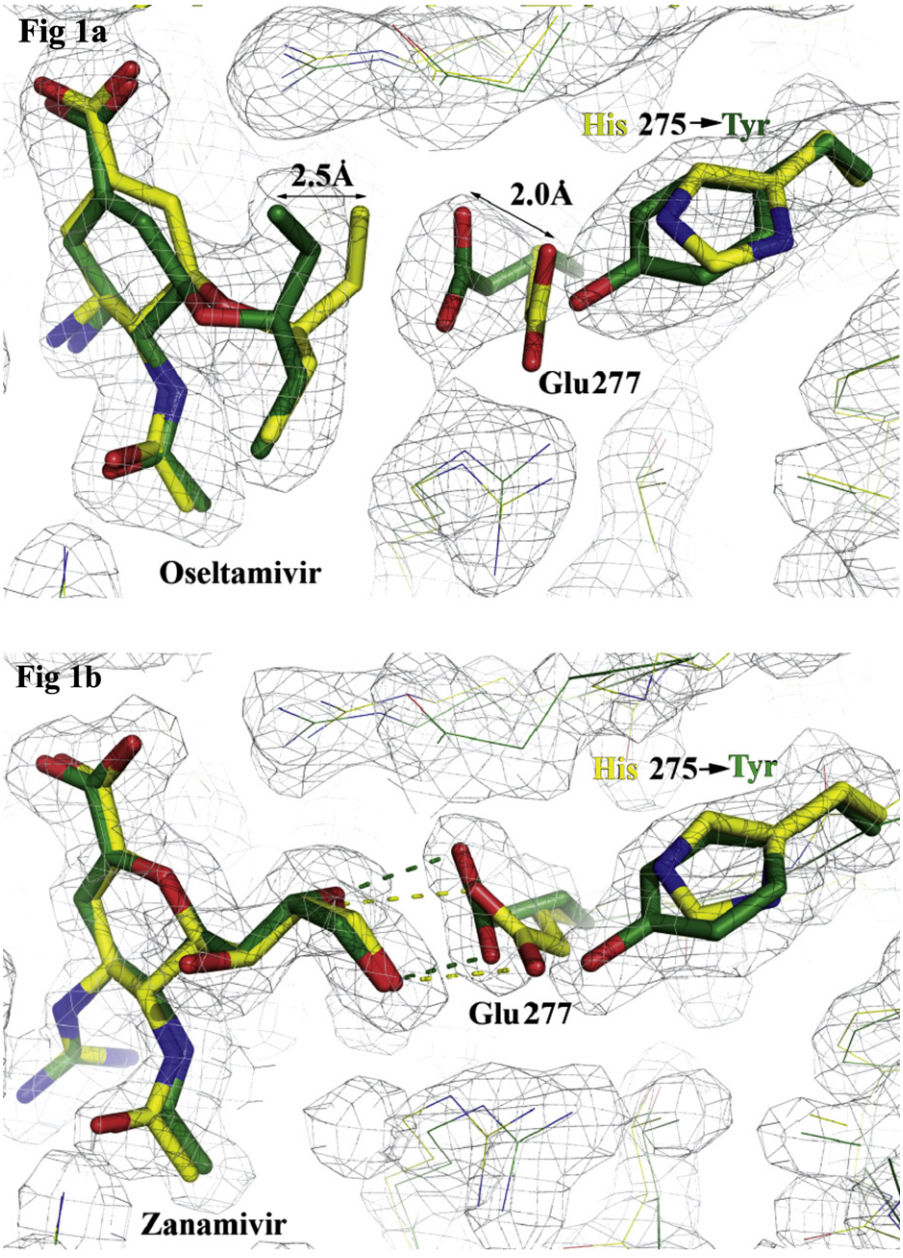

Fig. 1. X-ray crystallographic structures showing the effects of the H275Y mutation on interactions of oseltamivir (a) and zanamivir (b) with wild-type (yellow) and mutant (green) NAs of A/Vietnam/1203/2004 [14,15].