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Binding pattern of the long acting neuraminidase inhibitor laninamivir towards influenza A subtypes H5N1 and pandemic H1N1

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ABSTRACT

Influenza A H5N1 and pH1N1 viruses have broadly emerged and become widespread in various countries around the world. Oseltamivir, the most commonly used antiviral drug against the seasonal and pandemic influenza viruses, is targeted at the viral neuraminidase (NA), but some isolates of this virus have become highly resistant to this drug. The novel long-acting drug, laninamivir, was recently developed to inhibit influenza A and B viruses of either the wild-type (WT) or the oseltamivir resistant mutant of NA. To understand the high efficiency of laninamivir, all-atom molecular dynamics simulations were performed on the WT and H274Y mutant of H5N1 and pH1N1 NAs with laninamivir bound. As a result, the novel drug was found to directly interact with 11 binding residues mainly through salt bridge and hydrogen bond formation (as also seen by electrostatic contribution). These are comprised of 7 of the catalytic residues (R118, D151, R152, R224, E276, R292 and R371), and 4 of the framework residues (E119, W178, E227 and E277). Laninamivir showed a similar binding pattern to all four NAs, but strong hydrogen bonding interactions were only found in the WT strain, with a slightly lowered contribution at some drug contact residues being observed in the H274Y mutation. This is in good agreement with the experimental data that the H274Y mutant has a small increase (1.3–7.5-fold, which was not statistically significant) in the IC_{50} value of laninamivir.

1. Introduction

The avian flu (H5N1) and 2009 pandemic H1N1 (pH1N1) viruses are wide spread throughout the world and some isolates have become resistant to oseltamivir, the most common and (formerly) effective anti-influenza drug [1,2] (Fig. 1), leading to a potentially global public health problem. A new drug, laninamivir (R-125498), was approved and marketed in Japan in September 2010 [3]. With a long term inhibitory efficiency against the neuraminidase (NA) enzyme of influenza A and B viruses as well as their current oseltamivir resistant mutations [3–5], laninamivir is thus a very interesting drug not only for good efficacy but also in terms of a stockpile for future pandemic influenza. Although, the three dimensional structures of laninamivir binding to the wild-type (WT) NA subtypes N1 (from pH1N1, Fig. 2), N2 and N5 have recently been crystallized [6], the drug orientation and binding patterns towards the H5N1 virus and the H274Y (N2 numbering) mutant of both the pH1N1 and H5N1 viruses are not yet revealed. Therefore, the main goal of this present study was to provide a detailed comparative understanding of the drug–target interactions of laninamivir binding to the NA of both the WT and the H274Y mutant of these two N1 virus strains.

NA is a glycoside hydrolase enzyme (EC 3.2.1.18) that is found on the surface of the influenza virus and plays a pivotal role in the viral life cycle [7]. As such, NA cleaves the glycosidic bond of the terminal sialic acid from the host cell receptor with a new viral progeny release to infect other cells. Up to date, oseltamivir (Tamiflu®) and zanamivir (Relenza®), shown in Fig. 1, are the two common anti-influenza drugs targeted against, and operating as inhibitors of NA (NAIs). While oseltamivir is medicated as a tablet, zanamivir is administered by oral inhalation. Therefore, the oseltamivir is more commonly used to treat influenza patients. Another NAI drug, peramivir, was made available during the outbreak of the 2009 pandemic H1N1 virus until June 2010 under the Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA)

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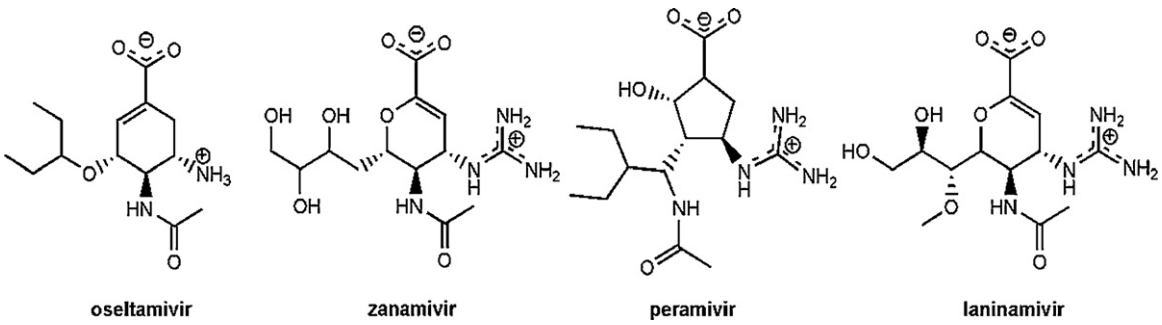

Fig. 1. The chemical structures of four NA inhibitors (NAIs) in the active metabolite form: oseltamivir, zanamivir, peramivir and laninamivir.

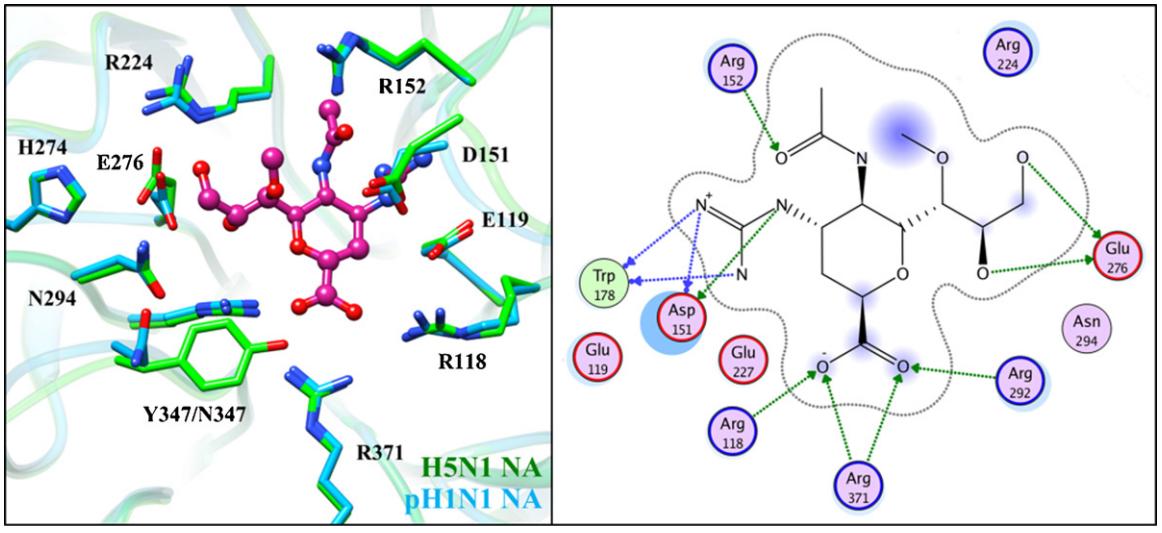

Fig. 2. (Left) Close up of laninamivir binding to the catalytic pocket of the pH1N1 NA (cyan) obtained from the 3TI3 crystal structure [6] with a superimposition of the H5N1 NA (2HU4 [15], green). (Right) From the X-ray structure of the laninamivir–NA complex, the hydrogen bond formations through the backbone and side chain of the surrounding residues are represented by blue and green arrows, respectively.

[8]. Recently, the second generation flu NAI drug, laninamivir, was discovered by Yamashita and co-workers [9] and manufactured by Daiichi Sankyo Co. Ltd. in Japan and Biota. It is an active metabolite converted in lung from the CS-8959 (laninamivir octanoate) prodrug [10,11]. Besides the long term efficient activity, with a single inhaled dose being comparable to that from taking oseltamivir twice daily for 5 days, laninamivir is effective against the wild-type strain of both influenza A (seasonal H1N1, pH1N1, H5N1 and H3N2) and B viruses as well as the oseltamivir-resistant H274Y mutations of H5N1, pH1N1 and seasonal H1N1 viruses [5,12,13] as summarized in Table 1. It is worth noting that the virus strain order, in terms of the fold increase in resistance to laninamivir, relative to wild-type is pH1N1 (1.36) < seasonal H1N1 (1.85) < H5N1 (3.44 or 7.50). In agreement with the experimental data, laninamivir and its prodrug were theoretically predicted to have high binding affinities with A/H5N1 complexes through the rupture forces and so that laninamivir could be used to inhibit both the WT and the oseltamivir-resistance providing N294S and H274Y mutations [14].

In order to understand the high susceptibility of NA from both the WT and H274Y mutant isolates of the influenza viruses towards the NAI laninamivir, molecular dynamics (MD) simulations were performed on the two influenza subtypes, H5N1 and pH1N1. The drug–target interactions, in terms of the hydrogen bonds, electrostatic and vdW forces, with the surrounding residues at the catalytic site of NA were intensively analyzed, discussed and compared with the co-crystal structure of pH1N1 NA with laninamivir bound, as well as with previous theoretical studies on the other anti-influenza NAI drugs.

2. Methodology

2.1. System preparation

All system preparations and MD simulations were performed using the AMBER10 software [16]. The four simulated systems of the WT and H274Y mutant of the NA from both the pH1N1 and H5N1 virus strains were prepared as follows. The starting structure of WT pH1N1 NA-inhibitor complex was taken from the recently determined co-crystal structure of the WT NA of pH1N1 virus with

Table 1
The 50% inhibitory concentration (IC_{50}) values of laninamivir for neuraminidase (NA) from various influenza strains.

Virus strain	NA change	IC_{50} (nM)	RF[b]
A/H1N1 (seasonal flu) [5]	WT	1.79	
pH1N1 [5]	WT	1.83	
A/H3N2 [5]	WT	2.13	
Influenza B [5]	WT	11–26	
A/Yogohama/67/2006 (seasonal H1N1) [13]	WT	3.03	
A/Yogohama/67/2006 (seasonal H1N1) [13]	H274Y	5.62	1.85
A/Washington/29/2009 (pH1N1) [12]	WT	1.57[a]	
A/Washington/29/2009 (pH1N1) [12]	H274Y	2.14[a]	1.36
A/Hanoi/30408/05 (HPAI H5N1) [13]	WT	0.32	
A/Hanoi/30408/05 (HPAI H5N1) [13]	H274Y	1.10	3.44
A/Vietnam/1203/04 (H5N1) [13]	WT	0.28	
A/Vietnam/1203/04 (H5N1) [13]	H274Y	2.10	7.50

[a] Fluorescein leakage (MUNANA) assay.
[b] RF = Resistance fold.