Efficacy of neuraminidase (NA) inhibitors against H1N1 strains of different geographical regions: an in silico approach

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Abstract This report described the efficacy of NA inhibitors against newly evolved strains of H1N1 viruses. This in silico study was designed to understand the mode of interactions of NA inhibitors with NA. Hence, ligand, oseltamivir, zanamivir and peramivir were docked with modeled NA, ACD65204 (USA/2007), BAA06717 (Japan/1992), ACE77988 (S. Korea/2005) and ACD65204 (USA/2007). This study is based on interaction energies. Ramachandran Z-scores for these modeled structures were found to be −0.998, −1.121, −0.870 and −1.023, respectively, which confirms the accuracy of the modeled structures. These interactions revealed that some of these interacting residues have remained conserved throughout all the pandemics. These amino acid residues were found to be R118, R152, R225, E277, E278, R293 and Y402. Moreover, our study concludes that peramivir is the most efficient inhibitor against NA of H1N1.

Keywords Neuraminidase · NA inhibitor · H1N1 · Swine flu

Introduction

A new H1N1 “swine” influenza virus was recently detected in individuals from the United States who presented respiratory symptoms, and the same virus was subsequently confirmed in patients from several countries around the world [1]. Influenza virus is a typical enveloped [2] negative-strand RNA virus which is composed of eight single-stranded genomic segments coding for 10 or more polypeptides [3, 4]. Influenza pandemics arise from genetic reassortment between avian and human influenza viruses or alternatively by the direct adaptation of an avian influenza viruses to efficient human-to-human transmission [5].

Designing of potential antiviral drug candidates in order to control influenza epidemics and eventual pandemics are the area of priority research. The most potential target to design inhibitors is neuraminidase (NA). These inhibitors act upon the active site of enzyme and stop releasing the newly assembled virions from the host cells to further infect cells of respiratory mucus. Two available NA inhibitors, zanamivir and oseltamivir have been found effective against seasonal influenza virus infection [6]. More inhibitors are in the stage of development like pyrrolidine-based compound from Abbott laboratories (A 315675) showing in vitro activity similar to that of zanamivir and oseltamivir against different strains (N1, N2, N9 subtypes) of influenza A viruses and B viruses [7, 8].

The emergence of drug-resistant variants is one of the disadvantages of antiviral therapy. There were reports on adamantane-resistant H1N1 and H3N2 influenza virus variants emerge just after treatment [9]. Moreover, zanamivir-resistant influenza virus variant have been isolated from only immunocompromised patients [10]. Only limited

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data is available on the appearance of variants with H274Y or N294S NA amino acid substitutions in five oseltamivir-treated patients with H5N1 infection [11, 12].

In view of the emerging problem of resistance against these inhibitors, we initiated to study the efficacy of these available inhibitors against H1N1 strains in different regions of the world during different pandemics.

Materials and methods

BLAST-P was performed to retrieve suitable templates for homology modeling using the study NA sequences which were in turn obtained from the 'NCBI Influenza resource'. Protein Data Bank (PDB) IDs of the templates retrieved for

modeling are shown in table 2. Modeling was performed using Swiss Model Server [13]. PDB structures of the NA-inhibitors were retrieved from Drug bank (<http://www.drugbank.ca/search/chemquery>). The ligand was docked into each of the modeled structures employing the Hex 5.1 software. Structural minimization of the whole complex was carried out until the final root-mean square deviation became less than 0.1 kcal/mol/Å°. The lowest-energy structures were selected as energy-refined complex models. The models were duly verified by Procheck [14] and verify 3D programmes [15]. Discovery Studio 2.0 (Accelrys) was used to prepare Ramachandran plots for the modeled structures. Ramachandran Z-scores for the evaluation of these models were calculated using 'WHAT IF' package. Alignments were performed by 'clustalW'.

Fig. 1 Neuraminidase modeled from sequence ACD65204 (USA/2007) docked with (a) oseltamivir, (b) zanamivir and (c) peramivir

Fig. 2 Interactions between modeled 'neuraminidase' and 'neuraminidase inhibitors'

- (a) Model from sequence ACD65204 (USA/2007) and Oseltamivir
- (b) Model from sequence ACD65204 (USA/2007) and Zanamivir
- (c) Model from sequence ACD65204 (USA/2007) and Peramivir

Fig. 2 (contd.) Interactions between modeled 'neuraminidase' and 'neuraminidase inhibitors'

- (d) Model from sequence ACE77988 (S. Korea/2005) and Oseltamivir
- (e) Model from sequence ACE77988 (S. Korea/2005) and Zanamivir
- (f) Model from sequence ACE77988 (S. Korea/2005) and Peramivir
- (g) Model from sequence BAA06717 (Japan/1992) and Oseltamivir
- (h) Model from sequence BAA06717 (Japan/1992) and Zanamivir
- (i) Model from sequence BAA06717 (Japan/1992) and Peramivir

Fig. 2 (contd.) Interactions between modeled ‘neuraminidase’ and ‘neuraminidase inhibitors’

- (j) Model from sequence ABV25644 (USA/1930) and Oseltamivir
 (k) Model from sequence ABV25644 (USA/1930) and Zanamivir
 (l) Model from sequence ABV25644 (USA/1930) and Peramivir

Table 1 Interaction energies in the docked complexes

Accession numbers of neuraminidase protein sequences used for modeling (NCBI Flu database)	Country/Year	Docking with oseltamivir			Docking with zanamivir			Docking with peramivir		
		E-total	E-shape	E-force	E-total	E-shape	E-force	E-total	E-shape	E-force
ABV25644	USA/1930	−214.28	−195.66	−18.63	−217.16	−156.25	−60.92	−270.5	−200.73	−69.76
BAA06717	Japan/1992	−209.65	−158.28	−51.38	−216.76	−163.28	−53.48	−294.63	−241.09	−53.53
ACE77988	S. Korea/2005	−217.36	−184.25	−33.11	−225.36	−165.02	−60.34	−268.59	−204.30	−64.28
ACD65204	USA/2007	−230.02	−165.85	−64.17	−205.93	−158.24	−47.69	−271.09	−200.42	−70.67

All energies are in KJ/mol

Results and discussion

We report here the efficacy of the available NA inhibitors against different strains of H1N1 virus. The rationale behind this study was to compare the emerging strains of H1N1 in different regions during different outbreaks for their resistance against antiviral therapy. The data was compared with the year of 1930 strain as an original strain. (Fig. 1) shows oseltamivir bound to neuraminidase (NA) modeled from sequence ACD65204 (USA/2007). Discovery Studio

2.0 analysis of the docked-complexes (Receptor: modeled NA; Ligand: Oseltamivir/Zanamivir/Peramivir) revealed that there are several amino acid residues which are crucial to the interaction between NA and NA-inhibitors (Fig. 2). Moreover, it was observed that some of these interacting residues have remained conserved throughout all the pandemics. These amino acid residues were found to be R118, R152, R225, E277, E278, R293 and Y402. The H1N1 strains possessing these NA sequences were reported in the years 1930, 1992, 2005, and 2007 and were isolated from USA, Japan, South Korea and USA, respectively.

It is noteworthy to mention that the choice of these NA-sequences was totally random with respect to year and country of isolation. A striking finding was observed during the clustalW alignment of these sequences, compared with the most recent NA sequence reported from Canada in 2009 pandemic (Fig. 3) that, the amino acid residues R118, R152, R225, E277, E278, R293 and Y402 were conserved in the Canadian sequence (Accession No.-ACR01027).

Interaction energies for the docked-complexes calculated by Hex5.1 are summarized in Table 1. A higher value of negative interaction energy is an indicator of more efficient interaction between the enzyme (NA) and its inhibitor. In terms of interaction energies, the order of efficiency for the study inhibitors against modeled NA sequences belonging to the years 1930, 1992 and 2005 was found as, peramivir (most efficient) > zanamivir > oseltamivir (least efficient). However, the trend for modeled NA sequence belonging to 2007 was found as, peramivir (most efficient) > oseltamivir > zanamivir (least efficient). A rise of resistance against oseltamivir in H1N1 viruses during 2007–2008 has been observed. Fifty seven of 896 influenza A (H1N1) strains had IC₅₀ S against oseltamivir ranging from 85.08nM to 255.83nM and are classified as extreme outlier [16]. Therefore, with such kind of data has become important to understand the molecular changes occurred in emerging strains H1N1 viruses.

Hence, Peramivir was found to be the overall most efficient inhibitor. Table 2 shows bioinformative details of the modeled neuraminidase structures. Sequences having accession nos. as ABV25644, BAA06717, ACE77988 and ACD65204 possessed 94.8%, 88.3%, 89.6% and again 89.6% sequence identity to the protein sequence used as template for homology modeling (PDB ID: 3b7Eb). Ramachandran Z-scores for these modeled structures were found to be −0.998, −1.121, −0.870 and −1.023, respectively. This confirms the accuracy of the modeled structures. Amino acid changes present in the NA sequences considering the

Fig. 3 ClustalW alignment of protein sequences (neuraminidase). 1, 2, 3, 4 and 5 represent accession nos. ABV25644, BAA06717, ACE77988, ACD65204 and ACR01027, respectively.

Table 2 Bioinformative details of the modeled neuraminidase structures

Accession Number (country/year) of neuraminidase protein sequences retrieved from 'NCBI Flu database'	ABV25644 (USA/1930)	BAA06717 (Japan/1992)	ACE77988 (S. Korea/2005)	ACD65204 (USA/2007)
PDB ID of the template retrieved for modeling	3b7eB	3b7eB	3b7eB	3b7eB
Modeled residue range	83 to 467	83 to 468	83 to 467	83 to 467
Sequence identity	94.805%	88.342%	89.61%	89.61%
E-value	0.00e-1	0.00e-1	0.00e-1	0.00e-1
Final total energy in KJ/mol (Modeling log)	−20488.779	−20141.658	−19681.824	−19518.279
Ramachandran Z-score	−0.998	−1.121	−0.870	−1.023

Table 3 Amino acid changes present in the neuraminidase sequences considering the sequence ABV25644 (USA; 1930) as a reference

Accession Number (country/year) of Neuraminidase protein sequences retrieved from 'NCBI Flu database'	Amino acid changes considering the sequence ABV25644 (USA; 1930) as a reference
BAA06717 (Japan/1992)	T3P, C14S, L15M, I16A, V17I, T20I, I34A, R41G, D42S, P46T, E47G, T48I, S52R, I53M, N59S, A72T, I74V, Q78K, A80K, M83V, I84T, R96G, S101T, I114V, T157A, I163L, S189G, N200D, D214E, N221K, K222Q, A232V, N248D, Q250P, K254R, R262K, I263V, I264T, N270D, S286G, K287T, D307N, N329K, T332E, G336N, S349T, S340V, N341D, N334D, F351Y, I365N, S366R, S367L, S369K, E382D, S386D, I393V, I396M, I418M, Q430R, K432R, Addition of K between E433 and N434 (with respect to the reference), N434T, V453A, G454N
ACE77988 (S. Korea/2005)	K6R, S12T, I13V, T20I, I23L, I30V, I32L, R41G, D42G, Q43K, P46T, T48M, S52N, I53V, E57V, Q64R, A72T, V75A, G77V, A80V, M83V, I94V, R95G, H126Q, S189G, A232V, V234M, I241V, M242L, I258V, R262K, N270D, S286G, G298A, I314M, T332K, S334N, S339L, F351Y, I365T, S366D, V389M, V394I, I396L, L415M, D416N, N434S, T438A, D451E, V453A, G454S, E462D
ACD65204 (USA/2007)	K6R, S12T, I13V, T20V, I23L, I30V, I32L, S35N, R41G, D42E, Q43K, N50S, S52N, I53V, A72T, A76D, D79G, A80V, M83I, R96G, K143R, S189G, A232V, V234I, I258M, R262K, I267V, N270D, S286G, M289V, G298A, S305T, D311N, T332K, S334N, S339P, V389I, V394I, I396L, L415M, D416N, R419K, N434S, N449D, D451E, G454S, E462D
ACR01027 (Canada/2009)	T3P, I13V, L15M, I16T, V17I, I19M, T20A, S21N, T40L, D42N, H45Q, P46I, I53V, A72T, I74F, V75A, D79S, A80V, T81V, M83V, I84K, I94V, S95G, R96E, Y100H, I106V, I114V, S123L, H126P, V149I, A166V, M188I, S189N, I241V, K257R, R262K, I264V, I267V, L269M, T285S, K287E, V288I, M289T, D307N, D311E, V321I, S328P, G331K, S334C, R352K, S369N, E382G, S385N, S386N, V389I, A395G, T397N, D398E, Q430R

sequence ABV25644 (USA; 1930) as a reference are shown in table 3. It is evident from the table that although there were a number of mutations present in the 2009 Canadian sequence (Accession No. ACR01027), amino acid residues R118, R152, R225, E277, E278, R293 and Y402 were still conserved. This information might be useful in designing future NA inhibitors for the rapidly mutating H1N1 strains.

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