In silico genome analysis and drug efficacy test of influenza A virus (H1N1) 2009

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Abstract The H1N1 2009 virus is pandemic in many countries. The genome of this virus contains eight segments. Among the eight segments maximum numbers of mutation occur at the segment 1 and segment 4 which codes for PB2 subunit and hemagglutinin (HA) and less number of mutations occur in segment 6 which codes for neuraminidase (NA) protein. Neuraminidase (NA) inhibitors (Oseltamivir and Zanamivir) are presently used as an anti-flu drugs. In the present study, the in silico efficacy of different drugs was tested against the swine flu virus. 3D structures of neuraminidase (NA) proteins of H1N1 2009 were generated using Geno3D. The 3D structure of H1N1 1918 was downloaded from PDB. Interaction study was done using Arguslab 4 and PyMol view. Oseltamivir and Zanamivir have good number of interactions with H1N1 2009 virus and the scoring function also support to this result. When compared with the 1918 H1N1 viral protein, 2009 H1N1 NA protein shows more number of interaction and good scoring function. The RMSD value of before and after docking are found to be same at $0.04\text{\AA}$ for both the drugs. The force field energy of NA protein 2009 was found to be -15603.529 KJ/mol before docking. The force field energy was found to be decreased after docking at -17620.740 KJ/mol with Tamiflu and -17652.242 KJ/mol with Zanamivir. The number of interaction and scoring function shows that Oseltamivir and Zanamivir will be able to effectively control the present pandemic H1N1 virus 2009.

Keywords H1N1 · Zanamivir · Oseltamivir · Neuraminidase · Hemagglutinin

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Introduction

In April 2009, the so called “swine flu” influenza A (H1N1) virus was first identified in Mexico and later it was identified in other American, European, African and Asian countries [1]. This virus is a major pathogen to human but not epidemic in pigs [2]. Influenza A viruses are negative sense single-stranded RNA viruses belonging to the family Orthomyxoviridae and possess a genome of eight single-stranded segments. Based on the antigenic properties of its surface glycoproteins, Influenza A virus is further classified as hemagglutinin (HA) and neuraminidase (NA). HA is of special interest due to its role in the viral entry mechanism and immune recognition. It consists of two subunits: HA1, which contains the receptor-binding and antigenic domains, and the HA2 subunit, which is responsible for the fusion of the virion with the endosomal membrane in the host cell [2]. All influenza viruses contain two surface antigens, namely hemagglutinin (H) and neuraminidase (N) and the viral strains are assigned as HxNx nomenclature based on which the forms of these two proteins are present. There are 16 H and 9 N subtypes known in birds, out of which only H-1, 2 and 3, and N-1 and 2 are commonly found in humans [3]. Out of the 144 total combinatorial possibilities of HAs and NAs only H1N1, H2N2 and H3N2 are human adapted virus [4]. The viral structure differs from strain-to-strain due to rapid genetic mutation in the genome. The newly identified strain H1N1 is responsible for swine flu in humans [3]. Influenza A H1N1 viral strain 2009 is a triple re-assortment of influenza flu viral strains: HA, NA, MP, NP and NS (originated from swine influenza virus); PB2 and PA (originated from bird influenza virus); PB1 (originated from human influenza virus) [5, 6]. Antiviral drugs such as M2 channel inhibitors (Amantadine and Rimantadine) and neuraminidase inhibitors (Oseltamivir and Zanamivir)

Table 1 Common mutations of H1N1 virus (2009). The eight segments of H1N1 genome of New York sequences (gi|242032105, gi|242032103, gi|242032101, gi|242032089, gi|242032096, gi|242032094, gi|242032091, gi|242032098) are taken as a representative sequence and aligned with the available H1N1 sequences in the NCBI sequence database using Blast algorithm. The segment 1 and 4 which codes for RNA polymerase and hemagglutinin are found to have maximum number of mutation.

Segment No.	Mutated region	Base pair changes
1	237	G → A
	915	C → T
	1105	G → A
	1233	G → A
	1465	G → A
	2022	T → G
2	377	A → G
	488	C → T
	629	A → G
3	421	G → T
	452	T → C
	1309	T → C
4	226	C → T
	277	A → G
	520	C → T
	521	C → T
	577	A → G
	579	A → C
	719	T → A
	991	G → T
	1186	G → A
	1767	A → G
5	43	A → T
	60	A → G
	864	T → C
	1446	C → G
6	21	T → C
7	17	G → C
8	17	G → T
	23	A → G

are widely used clinically to control this influenza virus. The function of M2 channel in the virus is to mediate an influx of H⁺ ions into the infecting virion early in the viral replication cycle, which facilitates the dissociation of the ribonucleoproteins from the virion interior and allows them to be released into the cytoplasm and transported into the cell nucleus [7]. High resistance to M2 channel inhibitors are found in the present H1N1 virus and also it is limited in use [2]. The resistance of M2 channel inhibitor is due to the mutation of S21N in the M2 channel and loss of the

Amantadine-binding site [8]. Some of the recent research report shows that Oseltamivir has resistance to the H1N1 virus but Zanamivir still effectively controls the pathogenicity of this virus [7, 8]. The Oseltamivir-resistant viruses are mainly isolated from adult in European swine flu cases [9]. Oseltamivir and Zanamivir are the neuraminidase inhibitor (NA) [7]. The H274Y substitution in the neuraminidase active site pushes the G276 further into the binding site and disrupts the hydrophobic pocket and leads to the resistance of Oseltamivir [7]. The incidence of influenza A (H1N1) viruses that carry the neuraminidase H274Y mutation has increased by 30% in this year in the Netherlands. Influenza A (H1N1) virus that carry this mutation is resistant to Oseltamivir but remains sensitive to Zanamivir [7]. But in the present study it is shows that Oseltamivir and Zanamivir will be able to effectively control the present pandemic H1N1 virus 2009.

Materials and methods

Genome analysis

The whole genome of the H1N1 virus (2009) is downloaded from NCBI (<http://www.ncbi.nlm.nih.gov/>). Genome of H1N1 virus consists of eight segments. The sequences were BLAST in the NCBI tool using New York sequence as representative sequence. From the BLAST result 100 sequences have been selected and multiple sequence alignment was performed using Clustal W in Bioedit. All the nucleotide segments of the genome were translated using GENSCAN (<http://genes.mit.edu/GENSCAN.html>). The translated protein sequences were run in InterProScan to check the protein signature, biological relevant sites and profiles in protein sequences.

Homology modeling of whole proteins

Comparative modeling is quite useful in predicting the unknown 3D protein structures by both starting from a known primary structure and relying on known 3D structures of homologous proteins. Sequentially related proteins are assumed to adopt similar conformations. The atomic positions in homologous regions are borrowed from known protein structures, while non homologous portions are predicted in various ways, including potential energy minimization, molecular dynamics and simulated annealing. Two most convenient criteria for similarity are: (a) an identity of at least 25% for a sequence size > 100 amino acids and (b) an expectation value (E) < 10⁻⁴, which gives the likelihood that similarities are due to chance [10].

Table 2 InterProScan analysis of H1N1 virus (2009). The table shows the protein signature, biological relevant sites and profiles in protein sequences of the H1N1 2009 influenza virus genome.

Segment No.	Protein function
1	RNA polymerase, PB2 subunit
2	RNA-dependent RNA polymerase, PB1 subunit, Influenza-type RNA-directed RNA polymerase, negative-strand RNA virus RNA-directed RNA polymerase, subunit PB1, Orthomyxoviridae type
3	RNA-directed RNA polymerase, Aldo/keto reductase, conserved site
4	Haemagglutinin, influenza A/B virus type, HA1 chain Haemagglutinin, influenza A/B virus type, HA1/HA2 chain Viral capsid/haemagglutinin protein Haemagglutinin, HA1 chain, beta-ribbon region Haemagglutinin, stalk Host cell surface receptor binding, signal-peptide
5	Influenza virus nucleoprotein (NP)
6	Glycoside hydrolase, family 34 Neuraminidase
7	Influenza matrix M1, N-terminal Influenza matrix M1, C-terminal Influenza matrix M1, N-terminal subdomain 1 Influenza matrix M1, N-terminal subdomain 2
8	Influenza non-structural protein (NS1) S15/NS1, RNA-binding

The H1N1 2009 has maximum identity of 90% with H5N1 neuraminidase (PDB id 2HTY). H5N1 NA protein is selected as a template for homology modeling. We used GENO3D an automatic online server to model the 3D structure. The quality of the modeled 3D structure is checked by using ProQ. The energy of the modeled protein was minimized in vacuum.

ArgusLab4/AScore/ShapeDock flexible docking

The docking problem is considered as a complicated optimization or an exhaustive search problem involving many degrees of freedom. The ultimate goal is to find out the optimal ligand/protein configurations with accuracy. At the same time we consistently predict their binding free energy without relying on formal statistical mechanical approaches. The A Score is based on the deconvolution of the total protein–ligand binding free energy. This is having several distinct components:

$$\Delta G_{\text{binding}} = \Delta G_{\text{vdW}} + \Delta G_{\text{hydrophobic}} + \Delta G_{\text{H-bond}} + \Delta G_{\text{H-bond(chg)}} + \Delta G_{\text{deformation}} + \Delta G_0$$

The dissected terms account for the van der Waals interaction between the ligand and the protein, the hydrophobic effect, the hydrogen bonding between the ligand and the protein, the hydrogen bonding involving charged donor and/or acceptor groups, the deformation effect, and the effects of the translational and rotational entropy loss in the binding process, respectively. The intraligand van der Waals energy is also included in the overall vdW term. These contributions can be conveniently written as the following products:

$$\Delta G_{\text{vdW}} = C_{\text{vdW}} x_{\text{vdW}},$$

$$\Delta G_{\text{hydrophobic}} = C_{\text{hydrophobic}} x HP,$$

$$\Delta G_{\text{H-bond}} = C_{\text{H-bond}} x HB,$$

$$\Delta G_{\text{H-bond(chg)}} = C_{\text{Hbond(chg)}} x HB_{\text{(chg)}}, \Delta G_{\text{deformation}} = C_{\text{rotor}} x RT \text{ and } \Delta G_0 = C_{\text{regression}}$$

Each of the contributions possesses a specific regression coefficient multiplying a term that has a clear physical meaning. By investigating the regression coefficients, it enables more profound insights into the receptor–ligand binding process [10].

The interaction of Oseltamivir and Zanamivir with the influenza A virus (H1N1 1918, and H1N1 2009) were studied after docking in Arguslab 4. The protein 3D structure of H1N1 1918 is downloaded from PDB. The 3D structures of 2009 H1N1 NA protein generated by homology modeling was used for docking. The docking results were saved in the PDB format and observed in the PyMol view. From the PyMol view it is able to find out the number of interactions present in the residue and distance of the hydrogen bonds.

Results

The available 2009 H1N1 genome sequence was downloaded from NCBI. It consists of eight nucleotide segments which were translated to eight protein sequences with the help of GENSCAN. These nucleotide sequences of the H1N1 2009 genome were analysed by NCBI-BLAST using New York H1N1 strain 2009 sequence as a representative sequence. BLAST result shows the diversity of different H1N1 virus sequences. By using multiple sequence alignment tool Clustal W these sequences were aligned and results show that only some of the segments are more susceptible for mutations in the H1N1 virus (2009). Summarized common mutations of H1N1 virus (2009) are given in the Table 1. For this analysis New York sequences were used

(A) Oseltamivir with 1918

(B) Oseltamivir with 2009

(C) 1918 Zanamivir

(D) 2009 Zanamivir

Fig. Illustrate the interaction of Oseltamivir (A, B) and Zanamivir (C, D) with H1N1 NA proteins of 1918 (A, C) and 2009 (B, D). Yellow dot line indicates the hydrogen bonding. In both the cases the number of interaction are increased in 2009 NA H1N1 protein. The scoring functions are also reducing in 2009 H1N1 with both the drugs. From the result we concluded that Zanamivir and Tamiflu will be effective in controlling the present pandemic H1N1 2009 influenza virus.

Table 3 Interaction of Tamiflu with the NA protein of influenza virus. The number of interaction is increased from four in 1918 H1N1 to seven in 2009 H1N1 neuraminidase protein. The scoring function or binding energy is also slightly reduced from -7.64 Kcal/mol in 1918 H1N1 to -7.8597 Kcal/mol in 2009 H1N1.

NA 1918 with Oseltamivir			
Residue	D-H-A	Distance (Å°)	Scoring function
Ser 287	O-H-O	2.79	−7.64 Kcal/mol
Ser 285	O-H-O	2.65	
Lys 350	O-H-O	2.43	
Try 317	N-H-O	3.00	
NA 2009 with Oseltamivir			
Tyr 320	N-H-O	2.55	−7.8597 Kcal/mol
Glu 196	N-H-O	2.91	
Arg 74	N-H-O	3.14	
Arg 74	N-H-O	2.64	
Arg 70	N-H-O	2.59	
Arg 70	N-H-O	2.84	
Arg 70	N-H-O	3.00	

as a representative sequence (gi|242032105, gi|242032103, gi|242032101, gi|242032089, gi|242032096, gi|242032094, gi|242032091, gi|242032098). The maximum numbers of mutations are found in segment 1 and segment 4 which codes for PB2 subunit and hemagglutinin. The result of the InterProScan is given in Table 2. InterProScan scans a range of protein signatures against the protein sequence (eight segments). These signatures mainly represent protein belongs to the same family, functional domains, or active site and can be used to extrapolate the potential function of query protein.

Interaction study of oseltamivir and zanamivir with the influenza A virus (H1N1 1918 and H1N1 2009)

The number of interaction and scoring function in 2009 NA protein was found to be more than 1918 H1N1. 1918 H1N1 NA has four interactions with Oseltamivir and six interactions with Zanamivir. But 2009 H1N1 NA protein has seven interactions with Oseltamivir and ten interactions with Zanamivir. The scoring function was also reduced in 2009 NA from -7.64 Kcal/mol in 1918 H1N1 to -7.8597 Kcal/mol with Oseltamivir and -7.44 Kcal/mol in 1918 H1N1 to -7.94953 Kcal/mol with Zanamivir.

The RMSD value of modeled 2009 NA protein of before and after docking of both the drugs were found to be 0.04 Å°. The force field energy of the modeled proteins was calculated. The energy is computed with a partial implementation of GROMOS69 force field. The force field energy of the 2009 NA protein was found to be reduced after

docking from -15603.529 KJ/mol to -17620.740 KJ/mol with Oseltamivir and -17652.242 KJ/mol with Zanamivir. The interactions of the drugs are shown in Tables 3 and 4. The figure shows the interactions of Oseltamivir and Zanamivir with NA proteins of H1N1 1918 and 2009.

Discussion

The in silico genome study reveals that majority of the mutations are taking place in segment 1 and segment 4 which codes for PB2 and hemagglutinin and only few mutations were found in the segment 6 which codes for neuraminidase (NA). Oseltamivir and Zanamivir (NA inhibitors) are presently used in controlling the present pandemic situation. The in silico efficacy of drugs Oseltamivir and Zanamivir were tested against the swine flu virus H1N1 1918 and 2009. The number of interaction and scoring function was found to be more in 2009 H1N1 NA protein (Tables 3 and 4; Fig). The result shows that both the drugs are able to effectively control the present pandemic H1N1 2009. Several recent studies reported that Oseltamivir is resistance to H1N1 virus of previous cases reported before April 2009 [8, 9,] and [11]. The resistance occurs due to the changes in the neuraminidase active site which leads to alter the binding pocket of the Oseltamivir, whereas changes accommodate Zanamivir without such an alteration in the binding pocket [12]. In the present study we found that both the drugs Oseltamivir and Zanamivir has good number of interaction and scoring function compare with H1N1 2009 to H1N1

Table 4 Interaction of Zanamivir with the NA protein of influenza virus. The number of interaction is increased from six in 1918 H1N1 to ten in 2009 H1N1 neuraminidase protein. The scoring function or binding energy is also slightly reduced from -7.44 Kcal/mol in 1918 H1N1 to -7.94953 Kcal/mol in 2009 H1N1.

NA 1918 with Zanamivir			
Residue	D-H-A	Distance (Å°)	Scoring function
Arg 70	N-H-O	2.91	−7.44 Kcal/mol
Lys 68	N-H-O	3.05	
Asp 117	O-H-O	2.98	
Asp 117	N-H-O	3.18	
Asp 117	N-H-O	3.21	
Asn 118	N-H-O	2.29	
NA 2009 with Zanamivir			
Tyr 144	N-H-O	2.68	−7.94953 Kcal/mol
Ser 98	N-H-O	3.09	
Trp 97	N-H-O	2.96	
Arg 143	N-H-O	2.35	
Arg 143	N-H-O	2.94	
Arg 74	N-H-O	2.74	
Arg 70	N-H-O	3.53	
Arg 70	N-H-O	3.19	
Arg 70	N-H-O	2.40	
Tyr 320	O-H-O	2.98	

1918. We concluded that Zanamivir and Oseltamivir will be effectively control the present pandemic H1N1 2009.

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