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Design of Novel Anti-Influenza Drugs that Circumvent Oseltamivir Resistance: A Critical Perspective

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Introduction

RRational drug design, as the multidisciplinary process of finding new medications based on the knowledge of the biological target, is arguably one of the most exciting scientific fields of our time. The major challenges in general are the improvement of the existing drugs and the creation of new types of drugs that allow treatment of diseases, such as cancer, AIDS, Parkinsonism, Alzheimer's disease, Influenza, against which no effective drug currently exists. Influenza is known for its enormous economic impact on population health. Of particular concern currently is a lethal avian influenza virus H5N1, known as "bird flu". The highly pathogenic avian influenza is most commonly found in migratory water birds. Water birds are generally resistant to infection caused by avian influenza, but the virus can cause severe disease when it spreads to poultry and other birds. The first cases of humans being infected by H5N1 were detected in Hong Kong in 1997. The key public health concern is to define the way in which the next influenza pandemic should be controlled. Vaccination is not a realistic plan for a rapidly spreading avian influenza pandemic. Vaccines require months to create, as it is necessary to identify a virus and grow antibodies against it. Besides, vaccine needs to be distributed, which takes time. A vaccine for the avian virus is being developed, but none is yet available. Thus, the question of vital importance is that in the absence of a specific avian flu vaccine, could antiviral drugs obstruct a pandemic? Should the virus spread from birds to humans [1]?

There are two classes of anti-viral drugs for treating influenza: adamantanes - M2 ion channel inhibitors and Neuraminidase (NA) inhibitors. Adamantanes (amantadine and rimantadine) are less expensive, more readily available, established as not being effective against most of the isolated avian flu viruses and their use can cause serious side effects such as seizures. Presumably, adamantanes would not be useful in an avian pandemic because of the capability of encouraging drug-resistant strains to emerge, while the strains can transmit from person to person. The class of neuraminidase inhibitors consists of the drugs Zanamivir (ZMV) and Oseltamivir (OTV), having the commercial names Relenza and Tamiflu respectively. If administered early before the virus infects too many cells, these drugs prevent the release of influenza virus from infected cells to healthy ones and their action is associated with very little toxicity. As neuraminidase enzyme acts at the final stage of infection, NA may thus be regarded as the key target for developing inhibitors that circumvent oseltamivir resistance. In other words, the design of new anti-influenza drugs is essentially the design of small molecules that are complementary in shape and charge to NA - the biomolecular target to which they are supposed to interact and therefore will bind to it.

Influenza A NA sequences are divided into two distinct phylogenetic groups. Although crystal structures of N1, N4 and N8 of group 1 and N2 and N9 of group 2 all have the same homotetrameric conformation, they display group-specific differences in the active site. The main conformational differences between the two groups were detected at the 150-loop (residues 147-152), adjacent to the active site [2,3]. These particular distinctions in the active site architecture were reflected through a 10×5×5 $Å^3$ 150-cavity adjacent to the active site in the group 1 NAs, which is not present in group 2 NAs. There is a difference of about 1.5 Å in position of the conserved Asp151 side chains and the carboxylate of the nearby conserved Glu119 points in approximately the opposite direction relative to that in group 2, resulting in a width increase of the active cavity by about 5 Å. The conserved Arg156 with the side chain approximately mid-way between Asp151 and Glu119 is located at the base of the 150-cavity and adopts almost the same position in the group 1 and group 2 NA structures, thus defining the entrance from the N1 active site into the 150-cavity. By examining the crystal structure of the avian influenza neuraminidase in complex with OTV (PDB ID: 2HU0B), known as the 'open' conformation of H5N1 NA:OTV complex, the possibility of developing novel inhibitors from the 4-amino group of OTV into the 150-cavity was proposed, while the prominent guanidinium side chain of Arg156 was identified as a prospective partner for a salt-bridge or hydrogen bond [2]. Despite the experimental proposal and the fact that some structural knowledge of all of the influenza proteins is now known, driven by the increased interest due to the threat of the next pandemic, the consistent understanding of the pivotal role of the 150-loop in developing inhibitors that circumvent OTV resistance is not quite clear. The objective is herein to put forward and reconcile several structural inconsistencies related to the most relevant structure of NA, as well as to elaborate some arguments of vital interest when envisioning new strategies for designing more effective and potent NA inhibitors relative to OTV.

The 'open' conformation of the crystal structure of H5N1 NA in complex with OTV (PDB ID: 2HU0) was used to rationalize the molecular mechanism of oseltamivir resistance using molecular docking simulations [4-6]. It was demonstrated that the 150-cavity can be exploited for designing the more potent inhibitors of H5N1 NA [4,7] in the same way as experimentally proposed [2]. The relevance of these observations [4-7] was questioned by pointing out both that an improper initial structure (PDB ID: 2HU0) was used and that a 'closed' conformation of the N1:OTV co-crystal structure is more appropriate for such studies [8]. Even though the discrepancies between the theoretical [5] and experimental [9] structures of the His274Tyr NA:OTV protein:ligand complexes were initially observed, the inclusion of protein flexibility, especially in the region of 150-loop, in the molecular docking protocol successfully fixed the glitch

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[10,11]. This quite a remarkable rationalization was in agreement with the previously proven considerable flexibility of the residues in the 150-loop of N1 from avian H5N1 [12]. Moreover, the possibility of designing more potent OTV derivatives than OTV itself was illustrated by both employing the 'open' conformation of the NA crystal structure (PDB ID: 2HU0) and taking advantage of the presence of the 150-cavity nearby the N1 NA active site [13]. It was concluded that the 'open' form crystal structure of 2HU0 provides a novel and reliable structural basis for rational drug design against influenza virus [13], thus speaking in favor of all the previous studies [4-7] being based upon the experimental proposal of Russell et al. [2].

This conclusion was substantiated by molecular dynamics simulations [12,14] focusing on the functional behavior of 150-loop in N1 from avian H5N1. Very flexible residues in the 150-loop essentially made the avian N1 protein capable of adopting a large variety of possible configurations in the 150-loop region [12]. Despite the fact that a closed 150-loop conformation in the same avian N1 NA was reported under particular experimental conditions [2], atomic-level structural insights showed that an open conformation of the 150-loop is favorable by the avian N1 overall [14]. Interestingly, the crystal structure of the 2009 H1N1 pandemic virus neuraminidase displayed the lack of 150-cavity in its active site [15], suggesting that the particular 2009 pandemic NA protein was structurally more comparable to the group-2 NAs than to the group-1 NAs. On the basis of alignments of sequences associated with all available NA crystal structures, the position 149 was hypothesized as critical for deter¬mining the (open or closed) conformational status of the 150-cavity, suggesting a new paradigm for comprehension of the presence of the 150-cavity in group 1 and in group 2 enzymes [14]. However, both OTV and ZMV are equally effective against both phylogenetic groups. The crystal structures of N1 NA in complex with ZMV or OTV revealed that the 150-loop can experience a conformational change upon inhibitor binding in such a way that the binding site of NA from the two groups is essentially identical when bound to an inhibitor [2]. This is the answer to why all of the inhibitors are effective against both phylogenetic groups in spite of their unliganded active sites having distinct conformations. Of note regarding the design of the next generation of NA inhibitors is that OTV can bind to N1 NA without giving rise to the conformational change of the 150-loop [2,3]. The 150-cavity can thus be targeted by new derivatives of OTV or ZMV in order to attempt to overcome the problem of drug resistance. The development of virus resistance to OTV fitting into NA active cavity was understood by way of conformational changes of the amino acids in charge of accommodating OTV's hydrophobic side and was viewed as a structural consequence of the prevention of the active site rearrangement due to the mutations Arg292Lys, Asn294Ser and His274Tyr [16]. Whereas such an active site rearrangement is not needed in the case of ZMV, structural studies of the influenza neuraminidase revealed that resistance to OTV would be more likely than that to ZMV [17]. The possibility of escaping the H5N1 NA mutants may thus be increased by maintaining the clear resemblance of new inhibitor candidates to sialic acid, a natural receptor from which ZMV is directly derived with minimal functional modifications [18]. It was shown that successful simultaneous modifications of the 4-amino side chain and bulky hydrophobic side chain of OTV are possible in order to concomitantly exploit experimentally identified potential benefits of the 150-cavity and maintain the clear structural resemblance of a novel inhibitor candidate to sialic acid [7]. In this context, both NA enzyme inhibition and X-ray crystallography data suggested that the strategy of designing an inhibitor of NA that binds to the highly conserved active site of the NA achieves the desired goal of activity against all influenza NA subtypes, N1-N9, and influenza B viruses [19,20].

Several critical arguments supporting the experimental proposal of Russell et al. [2] that the 150-cavity in avian influenza neuraminidase can be employed for designing novel NA inhibitors that circumvent OTV resistance are elucidated. The way in which the 150-cavity should be exploited is now lined up with the understanding that the 'open' conformation of the crystal structure of the complex of H5N1NA with OTV (e.g. PDB ID: 2HU0) represents a reliable structural basis for designing new anti-virals.

References

1. https://www.novapublishers.com/catalog/product_info.php?products_id=10457.
2. Russell RJ, Haire LF, Stevens DJ, Collins PJ, Lin YP, et al. (2006) The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. Nature 443: 45-49.
3. Kerry PS, Russell RJ (2009) The impact of structural biology on the understanding of the influenza virus and the rational design of antiviral. Nova Science Publishers Inc., New York,155-184.
4. Mitrasinovic PM (2010) Advances in the structure-based design of the influenza A neuraminidase inhibitors. Curr Drug Targets 11: 315-326.
5. Mihajlovic ML, Mitrasinovic PM (2008) Another look at the molecular mechanism of the resistance of H5N1 influenza A virus neuraminidase (NA) to oseltamivir (OTV). Biophys Chem 136: 152-158.
6. Mihajlovic ML, Mitrasinovic PM (2009) Applications of the ArgusLab4/AScore protocol in the structure–based binding affinity prediction of various inhibitors of group–1 and group–2 influenza virus neuraminidases (NAs). Molecular Simulation 35: 311-324.
7. Mitrasinovic PM (2009) On the structure-based design of novel inhibitors of H5N1 influenza A virus neuraminidase (NA). Biophys Chem 140: 35-38.
8. Rungrotmongkol T, Malaisree M, Udommaneethanakit T, Hannongbua S (2009) Comment on "Another look at the molecular mechanism of the resistance of H5N1 influenza A virus neuraminidase (NA) to oseltamivir (OTV)". Biophysical Chemistry 141: 131-132.
9. Collins PJ, Haire LF, Lin YP, Liu J, Russell RJ, et al. (2008) Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. Nature 453: 1258-1261.
10. Mitrasinovic PM (2009) Reply to Comment on "Another look at the molecular mechanism of the resistance of H5N1 influenza A virus neuraminidase (NA) to oseltamivir (OTV)". Biophysical Chemistry 141: 133.
11. Mitrasinovic PM (2011) Comment on 'Comment on "Another look at the molecular mechanism of the resistance of H5N1 influenza A virus neuraminidase (NA) to oseltamivir (OTV)"'. Biophysical Chemistry 154: 102.
12. Amaro RE, Minh DD, Cheng LS, Lindstrom WM Jr, Olson AJ, et al. (2007) Remarkable loop flexibility in avian influenza N1 and its implications for antiviral drug design. J Am Chem Soc 129: 7764-7765.
13. Wang SQ, Cheng XC, Dong WL, Wang RL, Chou KC (2010) Three new powerful oseltamivir derivatives for inhibiting the neuraminidase of influenza virus. Biochem Biophys Res Commun 401: 188-191.
14. Amaro RE, Swift RV, Votapka L, Li WW, Walker RC, et al. (2011) Mechanism of 150-cavity formation in influenza neuraminidase. Nat. Commun 2: 388.
15. Li Q, Qi J, Zhang W, Vavricka CJ, Shi Y, et al. (2010) The 2009 pandemic H1N1 neuraminidase N1 lacks the 150-cavity in its active site. Nat Struct Mol Biol 17: 1266-1268.
16. Moscona A (2005) Neuraminidase inhibitors for influenza. N Engl J Med 353: 1363-1373.
17. Moscona A (2008) Medical management of influenza infection. Annu Rev Med 59: 397-413.
18. von Itzstein M (2007) The war against influenza: discovery and development of sialidase inhibitors. Nat Rev Drug Discov 6: 967-974.
19. Roberts NA, Govorkova EA (2009) The activity of neuraminidase inhibitor oseltamivir against all subtypes of influenza viruses. Nova Science Publishers Inc., New York, 93-118.
20. Govorkova EA, Leneva IA, Goloubeva OG, Bush K, Webster RG (2001) Comparison of efficacies of RWJ-270201, zanamivir, and oseltamivir against H5N1, H9N2, and other avian influenza viruses. Antimicrobial Agents Chemother 45: 2723-2732.