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Review

Molecular dynamics simulations approaches for discovering anti-influenza drug

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ABSTRACT

The emergence of influenza virus and antigenic drift are potential cause of world-wide pandemic. There are some commercially available drugs in the market to treat influenza. During past decade, however, critical resistances have been raised for biological targets. Because of structural complexity and flexibility of target proteins, applying a computational modeling tool is very beneficial for developing alternative anti-influenza drugs. In this review, we introduced molecular dynamics (MD) simulations approach to reflect full conformational flexibility of proteins during molecular modeling works. Case studies of MD works were summarized for the drug discovery and drug resistance mechanism of anti-influenza pharmaceuticals.

Keywords influenza drug, molecular dynamics simulation, molecular modeling, protein structure

INTRODUCTION

As globalization develops, viral outbreaks spread quickly by international travel and often change to pandemic (Cox, 2000; Li et al., 2014). In particular, seasonal flu caused by three types of influenza virus become public health threats (Van Kerkhove et al., 2011; Duggal et al., 2016). During last ten years, 2009 influenza A (H1N1) outbreak was recorded as a pandemic which caused overall 16,000 deaths (Kannan and Kolandaivel, 2015). Because of their morbidity and mortality, development of suitable vaccination or therapeutics against influenza virus has been attempted by many research groups (Du et al, 2012). Oseltamivir and zanamivir are well-characterized approved drugs for treating some subtypes of influenza A and B (Jackson et al., 2011; McNicholl, 2011). These two drugs commonly inhibit biological activity of the viral neuraminidase which catalyzes terminal sialic acid from virus and from host cell receptor (von Itzstein, 2007). But drug resistances induced by antigenic drift demand also developing alternative drug candidates to preserve effective therapeutics for influenza infection (de Jong et al., 2006; Yamaya et al., 2014).

Identifying a lead compounds for the drug target is an important early stage to develop new therapeutic agent for the specific indication (Katsuno et al., 2015). For the influenza, both oseltamivir and zanamivir have originally developed by structure-based drug discovery process with transition state analogue for the neuraminidase. Because of its technical performance and economic feasibility, the structure-based molecule design has been mostly seen in the field of drug development during the few decades (Wang et al., 2016; Ö ster et al., 2015). In this review, we focused mainly on molecular dynamics (MD) simulation methodology to discover lead compounds for the influenza virus. The MD-based drug discovery is alternative approach to overcome conventional virtual screening process based on docking simulations.

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Molecular dynamics simulations for the drug discovery

Biomolecular MD simulations have been developed for studying a protein folding, enzyme activity, and ligand binding during past 40-years (McCammon et al., 1977; Salsbury, 2010). To conduct MD simulations, classical Newtonian equation are calculated for the every time-step to obtain force information (Radkiewicz and Brooks, 2000). Time-dependent atomic coordinates are traced by combination of physical force and initial structure originated from experimental analysis. During the process, every force change is reflected on a potential energy function of the molecules with force-field along the atomic coordinates (Salsbury, 2010). Currently AMBER (Case et al., 2005), CHARMM (Brooks et al., 1983), and GROMACS (Van Der Spoel et al., 2005) are the most popular force-field and associated with specific molecular modeling software to simulate structural characteristics of biomolecules. Molecular simulation is able to offer atomic detail of structure and motion concerning time-dependent changes. Therefore it can sample configuration space, approximate equilibrium state, and obtain actual dynamics with Boltzmann weighting (Karplus and McCammon, 2002). This is important advantage of the MD simulation compared to simple docking simulation approach used in virtual drug screening process. Since the typical docking simulations use rigid protein model, it is hard to reflect full-flexible protein dynamics in the computation (Mortier et al., 2015). For the MD, resulting trajectory file of ligand-receptor complex is analyzed to obtain structural and energetic information. A free energy estimation for the ligand-receptor complex can be processed by the MD trajectory analysis, in which free energy of binding is defined by energy difference between ligand free and bound to receptor in solution like a

following equation:

$$\Delta G^{bind} = G^{complex} - G^{free} = RT\ln K$$

A binding constant K between drug candidate and protein target can be easily derived from calculated free energy value by using MD simulations (Mortier et al., 2015). Thermodynamic integration (John and Kirkwood, 1935) and free energy perturbation (Zwanzig et al., 1954) are the most accurate and well-characterized free energy estimation method based on MD simulations. Recently, molecular mechanics (MM) with implicit water model has combined with MD simulations in order to reduce computational demand. A generalized Born surface area (GBSA) or Poisson-Boltzmann surface area (PBSA) is concise free energy calculating method (Wang et al., 2006; Kollman et al., 2000), in which binding energy is directly estimated from MD trajectory files using simplified equation like following:

$$G = E_{MM} + G_{solv} + G_{nonpolar} - TS_{MM}$$

where E_{MM} and S_{MM} is a potential energy and a system entropy from molecular mechanics at defined temperature. Each G_{solv} and $G_{nonpolar}$ is polar and nonpolar solvation free energy, respectively. In the MD simulations, sufficient equilibration is key step to calculate free energy and structural dynamics for the drug-protein system. This is reason for long CPU-time of MD simulation to obtain high-quality results, but this barrier is being overcome by improved sampling algorithm and fast computing resource (Lei and Duan, 2007). Next section we introduced successful case study on the developing anti-influenza drugs using by MD simulation approach.

Loop flexibility in the neuraminidase for anti-influenza drug design

The catalytic site of neuraminidase, primary target of anti-influenza drug, is observed to be comparatively rigid but having minor conformational flexibility. Russell et al discovered '150-loop' cavity (residue 147~152) adjacent catalytic site of influenza neuraminidase ten-years ago (Russell et al., 2006). They suggested the 150-cavity as a new drug target for development of neuraminidase inhibitors over oseltamivir or zanamivir. However, this alternative cavity was hard to determine as precise crystal structure because of 150-loop flexibility.

McCammon and coworkers in University of California San Diego recently have applied the MD simulation to obtain theoretical structure for the neuraminidase (Amaro et al., 2007). Through 40-ns of explicitly solvated MD simulations, they found active site of the neuraminidase could be expanded by motion coupling between neighboring 150- and 430-loop. This widely open conformation for the neuraminidase was clustered by Cheng et al using MD simulations with AMBER force field (Cheng et al., 2008). From extensive MD simulations, multiple neuraminidase structures were collected as an ensemble which was used for receptor structures for virtual drug screening process by docking simulations with 2000 compounds in NCI library collection. This ensemble-based virtual screening approach has outperformed simple crystal structure-based virtual screening. After ensemble-based virtual screening finally 27 hit compounds were discovered, and half of which were ranked to be inactive if conventional crystal structures are used. Derived hit molecules were widely located at different binding sites of neuraminidase including catalytic, 150-, and 430-cavity. They suggested that using MD-generated structures broader range of receptor configurations could be systemically incorporated into the hit identification procedure.

Lu and Chong have studied free energy of binding for 20 flavonoid derivatives with neuraminidase N1 using MD simulations (Lu and Chong, 2012). The conducted docking simulations to obtain complexed pose of each flavonoid derivative with N1 structure, and followed by 10-ns of MD simulations for each complex. From the trajectory analysis, Trp179 and Tyr402 in the N1 were critical to hydrophobic contribution of inhibitor binding whereas Arg152 and Asn295 were important residues for a hydrophilic affinity. In particular hydrophilic nature was critical to determine inhibitory activity of the flavonoid derivatives because the binding affinities were semi-quantitatively increased by the presence of hydroxyl and oxygen moieties.

Drug resistance mechanism of aminoadamantanes for the influenza M2 channel

M2 channel is a viral membrane protein having H+ selectivity to induce protein influx into the virus interior during infection process (Pinto et al., 1992; Wang et al., 1995). Commercial drugs to block M2 channel has used as inhibitors for replicating an influenza type-A (Hay et al., 1985). However, over 90% of influenza A has developed drug resistance for the M2 blocking amino-adamantyls such as amantadine and rimantadine (Bright et al., 2006). In particular mutations at Ala30 and Ser31 was mainly responsible for the drug resistance by decreasing a binding constant (Astrahan et al., 2004). In 2011, Leonov et al published MD simulations results on the M2 channel blocking by aminoadamantyls (Leonov et al., 2011). They explained drug resistance mechanism as a result of changes in drug mobility in the M2 channel. In this model, both Val27 and Ser31 were key residues determining drug binding and mobility. The wild-type M2 channel was suitable for maintaining a drug binding to immobile state. Moreover, charged amine group in the adamantyls enforced a positive potential to repel protons from inner space of the channel. For the S31N mutant, adamantyls were hardly bound during MD simulations because of steric hindrance caused by Asn31. In contrast, adamantly drug was able to bind to V27A mutant, in which Ala residue was much smaller than the Val. However, increased pore size by V27A mutation exert drug to sufficiently mobile to hinder biological activity. That suggestion was mainly made by theoretical view from the MD simulations for aminoadamantyls and M2 channels.

MD simulation studies on the oseltamivir-resistant mutants of influenza neuraminidase

Some kinds of influenza mutants have been identified in the oseltamivir-resistant mutations such as N294S and H274Y in the neuraminidase (Kiso et al., 2004; Collins et al., 2008). Those mutations were critically emerged in the viral strain H1N1, H3N1, and H5N1. Since drug-resistant strains can lead to pandemic outbreak, understating a molecular mechanism for the oseltamivir-resistance is important to improve drug efficacy. Hou and coworkers performed 70-ns of MD simulations for the oseltamivir-resistance of 2009 A/H1N1 strain (Li et al., 2012). They characterized differences in specific interactions of oseltamivir for wild type neuraminidase and for point-mutants including H274Y, N294S, and Y252H. To evaluate oseltamivir binding into neuraminidase, they used free energy

decomposition analysis after doing series of MD simulations. From MM/PBSA and MM/GBSA analysis, they predicted inhibitory potency of oseltamivir to the neuraminidase as ordered by wild type = Y252H > N294S > H274Y. Therefore it was consistent with experimentally determined results, in which oseltamivir was resistant to the N294S and H274Y mutants of neuraminidase. In particular polar contributions of free energy of binding were dominant factor to cause drug resistance.

A similar study for the oseltamivir resistance was performed by Woods et al using long time-scale MD simulations (Woods et al., 2012). They conducted 1-μs of MD simulations for the I223R/H275Y mutated neuraminidase complexed with oseltamivir to designate drug resistance. The computational results demonstrated that binding affinity of the oseltamivir was reduced by conformational changes of the neuraminidase to the opened cavity adjacent 150-loop. Next year they published successive research results on the oseltamivir-resistance by using multiple 500-ns of MD simulations (Woods et al., 2013). In this work, drug-binding/unbinding to the neuraminidase was decisive factor to affect resistance. In particular oseltamivir unbinding was driven by competitive interaction between R222 and R151 residues on the 150-loop of the mutant. They also observed that water dynamics in the active site was key point to understand thermodynamics and kinetics for the binding of oseltamivir.

CONCLUSION

In current drug discovery process, MD simulations have many roles in conventional virtual screening, protein structure analysis, and small-molecule design. Both protein flexibility and structural diversity is potential hurdle to design a drug candidate for the influenza. To develop anti-influenza drugs, MD simulations will become practical methodology because of conformational flexibility of the drug-target proteins.

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CONFLICT OF INTEREST

The author states no conflict of interest.

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