Interaction of Andrographolide with Neuraminidase of H5N1 Virus and Nuclear Factor-kappaB

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Abstract
Andrographolide is an active compound of *Andrographis paniculata* (Burm.F) Nees, which was used empirically as medicine plant. This compound was a major constituent that was extracted from *Andrographis paniculata* (Burm.F) Nees leaves. The aim of this project was to understand the interaction of andrographolide with H5N1 neuraminidase and NF-kappaB at molecular level by docking the compound into the active sites of the macromolecules. The docking method was validated by redocking several known inhibitors into the origin binding pocket of the macromolecules. Scoring calculation, using London dG scoring function, of andrographolide with the DNA binding region of NF-kappaB compared to aurine tricarboxylic and gallic acid are -9.99 kcal/mol, -12.18 kcal/mol, and -12.45 kcal/mol, respectively, while its scoring calculation with H5N1 neuraminidase compared to oseltamivir and zanamivir were -10.04 kcal/mol, -10.84 kcal/mol, and -12.48 kcal/mol, respectively. This study indicates that andrographolide is able to be proposed as inhibitors of NF-kappaB and H5N1 neuraminidase. This compound interacts with H5N1 via hydrogen bonds and van der Waals interactions. The pharmacophores of andrographolide were the lactone ring and the hydroxyl group.

Keywords: Andrographolide, H5N1 virus, neuraminidase, NF-kappaB, molecular modeling study

Interaksi Andrografolid dengan Neuraminidase Virus H5N1 dan *Nuclear Factor*-kappaB

Abstrak
Andrografolida merupakan senyawa aktif *Andrographis paniculata* (Burm.F) Nees, yang digunakan secara empiris sebagai tanaman obat. Senyawa ini merupakan konstituen utama yang diekstrak dari daun *Andrographis paniculata* (Burm.F) Nees. Tujuan penelitian ini adalah untuk memahami interaksi andrografolida dengan neuraminidase H5N1 dan NF-kappaB pada tingkat molekul dengan *docking* senyawa ke dalam situs aktif dari makromolekul. Metode *docking* divalidasi oleh *redocking* beberapa inhibitor yang dikenal ke dalam *origin binding pocket* dari makromolekul. Perhitungan nilai, menggunakan fungsi penilaian London dG, andrografolida dengan wilayah yang mengikat DNA dari NF-kappaB dibandingkan aurin trikarboksilat dan asam galat masing-masing adalah -9,99 kkal/mol, -12,18 kkal/mol, dan -12,45 kkal/mol, sementara perhitungan nilai neuraminidase H5N1 dibandingkan dengan oseltamivir dan zanamivir masing-masing -10,04 kkal/mol, -10,84 kkal/mol, dan -12,48 kkal/mol. Penelitian ini menunjukkan bahwa andrografolida mampu diusulkan sebagai inhibitor NF-kappaB dan H5N1 neuraminidase. Senyawa ini berinteraksi dengan H5N1 melalui ikatan hidrogen dan interaksi van der Waals. Farmakofor dari andrografolida adalah cincin lakton dan kelompok hidroksil.

Kata kunci: Andrografolida, neuraminidase, NF-kappaB, studi pemodelan molekul, virus H5N1

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Introduction

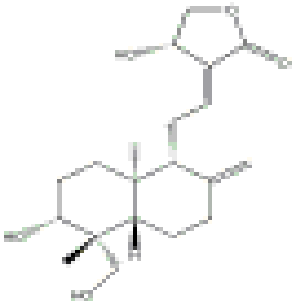

Figure 1 Structure of Andrographolide[1]

One of *Andrographis paniculata* (Burm.F) Nees's active compounds is andrographolide. It is a major constituent that is extracted from leaves, and it is empirically used as medicine plant.[2]

Andrographolide's activities have been reported by some researches, which can be used as antiinflammatory.[3 – 5] Not only as antiinflamatory, andrographolide also have antihyperglicemia activity and antioxidant activity.[6] Andrographolide can inhibit the proliferation of T-cell and release cytokine as a response to allogenic stimulation.[7] In dose of andrographolide was 100 mg/kg bodyweight of rats showed that it's has antioxidant activity and can decrease lipid peroxidation.[6]

Nuclear factor-kappa B (NF-kappaB) beginning with its discovery by Baltimore (1986) and continuing to the present, has attracted widespread interest based on its unsual regulation, the kind of stimuli that activate it, biological responses that it controls, the diverse genes and the striking evolutionary conservation of structure and function among family members, and its apparent involvement in a kind of human diseases. Importantly, consistent with the last point, NF-kappaB has been shown to be the target of several antiinflammatory and anticancer drugs.[8]

NF-kappaB is inducible transcription factor of Rel family and it is sequestered in the cytoplasm by the IkappaB family of proteins. NF-kappaB can exists in several dimeric forms, but the predominant one is p65/p50 heterodimer. Activation of NF-kappaB by wide range of stimuli including viral products and oxidative stress, leads to phosphorilation and proteasome dependent degradation of IkappaB, lead to the release of free NF-kappaB. This free NF-kappaB then binds to its target sites in the DNA to initiate the transcription.[9] These kappaB sites are also present in the Long Terminal Repeat (LTR) of HIV-1, and hence NF-kappaB (p50 subunit) binding to LTR-DNA is critical poin in viral replication.[10] Targeting direct p50-DNA binding, in this regard, is a recent approach of antiHIV gene expression inhibitors design, which do not have problem of resistance unlike in other antiHIV strategies.[11]

A palindromic kappaB site is bound the 2.3 Å crystal structure of the transcription factor NF-kappaB p50 homodimer (Figure 2a) reveals that the Rel homology region folds into two distinct domains, similar to those in immunoglobulin super family. The p50 dimer envelopes an undistorted B-DNA helix, and making specific contacts along the 10 base pair kappaB recognition site mainly pass through loops connecting secondary structure of elements in both domains. The carboxy terminal domains form a dimerization of interface between beta-sheets using residues that are strongly conserved in the Rel family.[12]

Influenza pandemics, a global outbreak of the disease due to viruses with novel antigenic subtypes, have caused death on human population. Pandemics were caused by the hybrid viruses that contained avian and human viral genes. Main contributors of human influenza pandemics are avian influenza viruses.[13]

Influenza viruses, which belong to the *Orthomyxoviridae* family, are classified as A, B, and C based on antigenic differences in their nucleoprotein (NP) and matrix (M1) protein. All avian influenza viruses are classified as type A. Further subtyping is based on the antigenicity of two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Nowadays, 16 HA and 9 NA subtypes have been identified among influenza A viruses.[13]

(a) (b)

Figure 2 3D Structure of NF-kappaB p50 Homodimer[12] (a), H5N1 Neuraminidase[16] (b)

Two glycoprotein spikes, HA and NA, and the M2 protein are embedded in the lipid bilayers derived from the host plasma membrane. HA elicits virus neutralizing antibodies. NA is a sialidase that prevents virion aggregation by removing cell and virion surface sialic acid.[14]

Hence NA is antiviral potential target due to its key role in the infection step of the virus to other cells.[15] By inhibiting NA, the virus will not be able to move outward from the cell and finally it will be die.[16]

The interaction of andrographolide with NF-kappaB and H5N1 neuraminidase (Figure 2b) can be studied by molecular modeling technique, example molecular docking. The focus of molecular docking is to get the optimal conformation, both for the protein and the ligand. In the case of molecular docking, particular arrangement of a ligand and a protein can be defined by a set of values describing the translation, orientation, and conformation of the ligand with respect to the protein. These are the ligand's state variables and, in the genetic algorithm, each state variable corresponds to a gene. The ligand's state corresponds to the genotype, the other way the phenotype corresponds to its atomic coordinates. In molecular docking, the fitness is the total interaction energy of the ligand with the protein, and is evaluated using the energy function.[17]

Methods

Instruments that used for this study were personal computer with Mobile Dual Core Intel Core 2 Duo T7250, 1978 MHz (10x198) (www.intel.com), harddisk 250 GB, graphic ATI Mobility Radeon HD 3400 Series (256 MB) and system memory 2044 MB (DDR2-667 DDR2 SDRAM). Softwares used in this study were MOE 2007.09 (Chemical Computing Group, www.chemcomp.com), and HyperChem v7.03 (www.hyper.com).

The research start with macromolecule preparation. The 3D structures of NF-kappaB crystallized using X-ray diffraction by Gosh G, et al.[8] The resolution is 2.30 Å, downloaded from online PDB (Protein Data Bank) code 1NFK, chain A was selected and getting seperated from the macromolecule by using MOE sequence editor. Hydrogens were added to the macromolecule PDB crystal structures followed by calculating the partial charges by using Amber 99. Site finder tool was held to every crystal structure molecule to predict the most likely sites for the ligand-macromolecule interactions. The amino acid sequence in the macromolecules was analized using MOE sequence editor.

3D structures of neuraminidase H5N1 cocrystallized with zanamivir (PDB access code: 2HTQ, resolution 2.2 Å crystallized by Russell RJ).[16] Neuraminidase H5N1 cocrystallized with oseltamivir (the PDB access code: 2HT8 resolution 2.6 Å) were downloaded from online PDB.[12]

The 2D structure of andrographolide was copied from Fujita.[18] The 2D structure built using MOE-2007.09.02 builder, 2D structure of aurine tricarboxylic acid and gallic acid copied from Vineet Pande.[11]

(a) (b)

Figure 3 The Electrostatic Map of Andrographolide (a), Pharmacophores of Andrographolide [Showed as Circles] (b)

The 2D structure then built by using MOE-2007.09.02 builder. Energy minimization of each molecule was carried out by using MMFF94x a molecular mechanics force field. Stochastic conformational search with molecular mechanics method was held to find the lowest energy conformer.

The interaction of andrographolide with the macromolecules was analyzed and also compared with the inhibitors. Docking was performed by London dG scoring function and Alpha Triangle placement method.

Results

The andrographolide pharmacophores and also the result electrostatic map of andrographolide showed in Figure 3. The visualizations docking of andrographolide with DBR of p50 NF kappaB and neuraminidase showed in Figure 4. The scoring calculations from andrographolide with p50 NF-kappaB and neuraminidase showed in Table 1.

Discussion

3D structures of the compounds were built by using MOE-2007.09.02 builder, which constructed the molecules from preexisting fragment libraries. Fragment libraries can be utilized like an electronic 3D tool kit, which is easy to handle. Because of the preoptimized standard geometries of all entries in the fragment pool, the resulting 3D structures already have an acceptable geometry. Mostly only torsion angles have to be cleared to avoid atom overlapping or close van der Waals contacts.

Molecular structures generated using preexisting fragment libraries described previously should always be geometry optimized to find the individual energy minimum state. This is normally done by applying a molecular mechanics method. The molecular mechanics considers the

(a) (b)

Figure 4 Visualization of the Docking of Andrographolide with DBR of p50 NF kappaB (a) and Neuraminidase (b)

atomic composition of a molecule to be a collection of masses interacting with each other via harmonic forces. As a result of this simplification, molecular mechanics is a relatively fast computational method practicable for small molecules as well as for larger molecules and even systems of oligomolecular. In the calculation, the total energy is minimized with respect to atomic coordinates, where:

$$E_{total} = E_{str} + E_{bend} + E_{tors} + E_{vdw} + E_{elec} + \ldots$$

E_{total} is total energy of the molecule, E_{str} is bond stretching energy term, E_{bend} is angle bending energy term, E_{tors} is torsional energy term, E_{vdw} is van der Waals energy term, and E_{elec} is electrostatic energy term.

In minimization procedure, molecule structure will be relaxed. Therefore, all of the above corresponding energy terms are altered in a force field optimization.

Molecules are not rigid. At room temperature, the motional energy is large enough to let all atoms in a molecule move permanently. A stochastic conformational search using molecular mechanics method of MOE-2007.09.02 software was held to find the individual energy minimum state of andrographolide and known inhibitors of NF-kappaB conformational search produces eleven conformers. The molecule is flexible due to its many single bonds.

The andrographolide structure shows that the molecule is likely form hydrogen bonds considering its carbonyl, lactone and hydroxyls (see Figure 1). Andographolide is a lipophylic compound which has log P value 2.24 (calculated by using Hyperchem Release7).

The docking was performed by London dG scoring function and Alpha Triangle placement method. Andrographolide can interacts with DBR p50 NF-kappaB via four hydrogen bondings with Asp336, Arg51, Asp206, and Asn244 with its scoring value is -9.99 kcal/mol and the scoring value describes its docking energy.

The same interactions are also showed by aurine tricarboxylic acid and gallic acid, known inhibitors of p50 NF-kappaB. This docking indicates that andrographolide is potential to be developed as inhibitor of

Table 1 Scoring Calculations Andrographolide with p50 NF-kappaB and Neuraminidase

Ligand	Scoring [kcal/mol]	Macromolecule target	Hydrogen bonding (donor→acceptor)
Aurine tricarboxylic acid	-12.18	p50 NF-kappaB	Arg51→ O OH → Ser208 OH → Asn244 O → Asp336
Gallic acid	-12.45	p50 NF-kappaB	Arg51→ O Ser208 → O OH → Asn244 OH → Asp336
Andrographolide	- 9.99	p50 NF-kappaB	Arg51→ O OH → Asp206 OH → Asn244 OH → Asp336
Andrographolide	-9.64	Neuraminidase	Tyr347 → O Arg371 → O
Oseltamivir	-10.84	Neuraminidase	Arg118 → O Arg152 → NH Arg292 → O Arg371 → O
Zanamivir	-12.48	Neuraminidase	Arg118 → O Arg152 → NH Arg292 → O Arg371 → O

According to both interactions with p50 NF-kappaB and neuraminidase, the pharmacophores of andrographolide are the lactone ring and the hydroxyls attached to C-14 and C-19 as showed in Figure 3b.

Conclusions

From this research can be concluded that andrographolide is able to be proposed as inhibitors of NF-kappaB and H5N1 neuraminidase. This compound interacts with H5N1 via hydrogen bonds and van der Waals interactions. The lactone ring and hydroxyl group are the pharmacophore of andrographolide.

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