In silico development of new candidate of NADPH oxidase inhibitor for hypertension treatment

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Abstract. Hypertension is a silent killer that becomes the important risk factor for stroke and ischemic heart disease. Oxidative stress which results from high production of reactive oxygen species (ROS) in the endothelial layer, contributes to hypertension pathophysiology. The high activity of NADPH oxidase is the main ROS source. Many medicinal plants have been developed to treat some human diseases. This study aimed to explore virtually Indonesian phytochemicals as a NADPH oxidase inhibitor for hypertension treatment. This bioinformatics study used a molecular docking method with P47-phox protein and apocynin as protein target and standard ligand respectively, which were obtained from Protein Data Bank and ZINC databases with 1NG2 and 0162515 codes. Indonesian phytochemicals were obtained from the HerbalIBD, had molecular structure from the PubChem database, and met the Lipinski’s criteria. The AutoDock Vina version 1.1.2 was used to analyse the binding affinity and sites and the PyMol 1.3 program was for visualization of molecular docking results. Apocynin interacted with P47-phox with -5.5 kcal/mol binding score and binding at Asp221, Arg302, Arg316 residues to prevent NADPH activation. Compared to apocynin, morindone had lower binding score (-7.7 kcal/mol) to bind to P47-phox and had similar binding sites at Arg 302, Arg 316, and Arg 318 residues. In conclusion, morindone potentially becomes a NADPH oxidase inhibitor in silico for hypertension treatment.

1. Introduction

Hypertension is a global health problem in the world with high prevalence and increase risk of cardiovascular disease. About 7.5 million or 12.8% from whole death in the world caused of high blood tension [1] Hypertension defined as a condition when blood pressure systole reaches ≥140mmHg and diastole blood pressure reaches ≥90mmHg [2]. The pathophysiology of hypertension may result from excessive production of reactive oxygen species (ROS), namely oxidative stress because of nitric oxide (NO) degradation and lack of antioxidants in the heart, blood vessel, kidney and brain [3,4,5]. The majority of ROS production is related to nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [6]. The NAPDH oxidase (Nox) Family comprises Nox 1, Nox 2, Nox 4 and Nox 5 members, which play an important role in cardiovascular diseases [7] but the last Nox family member is not related to hypertension [8].

In recent year, apocynin has been developed as a NADPH oxidase inhibitor, which is able to reduce blood pressure in rat model with hypertension [9]. The action mechanism of apocynin inhibits
phosphorylation of p47-phox, leading to inactivation of Nox 1, Nox 2, Nox 4 and Nox isoform [10,11]. Virtual Screening through molecular docking is one method for screening new drug candidates. Theoretically, molecular docking can be used to search and to predict efficiently complex structures between two molecules in silico because it needs short time and spends less money, compared to in vitro or in vivo study [12].

Indonesia has thousands of medicinal plants that contain various active compounds and is potentially developed as herbal medicines [13]. For example, a secondary metabolite in the Tectona grandis plant has been proven as in silico drug for gestational hypertension [14]. Therefore, this study aimed to conduct molecular docking between phytochemicals from Indonesian herbal plants and P47-phox protein for development of anti-hypertension drug through NADPH oxidase inhibitor.

2. Methodology

This bioinformatics study used the molecular docking method. Three dimensional structure of P47-phox protein was downloaded from Protein Data Bank (PDB) with 1NG2 access code. Apocynin is the standard ligand which was downloaded from Zinc database with 0162515 access code. The samples of this study were Indonesian phytochemical compounds that were registered in the HerbalIDB database, registered in PubChem and met with Lipinski's Rule of Five criteria. The three-dimensional structure was downloaded from the PubChem National Center for Biotechnology (NCBI). The software used is AutoDock Vina version 1.1.2 which is used to analyze bond energies and PyMol 1.3 for visualization of molecular docking results.

This study had several stages. First searching and downloading three dimensional structure from P47-phox, Apocynin and Indonesian phytochemical plant which met with Lipinski's Rule of Five criteria. Second, processing molecular docking between p47-phox p47-phox receptor and Indonesian herbal phytochemical plant with AutoDock software. Third, analyzing bond energy by comparing to the bond energy of the p47-phox-apocynin receptor. Fourth, analyzing the location of the bond by visualizing the results of molecular docking using the PyMol program. Finally, look for herbal phytochemicals that have a more negative bond than apocynin and have the same binding site with P47-phox as apocynin. So this phytochemical has the possibility to inhibit NADPH oxidase activity through P47-phox.

3. Result and Discussion

3.1 Target Protein and Ligand Preparation

Target protein will be prepared first before docking in AutoDock Vina to exclude effect of hydrogen bond from macromolecule. Three dimensional target structure was downloaded from PDB with code 1NG2. Macromolecule p47-phox has inhibition region to bind with p22-phox at SH3 and argine/lysine rich region [PBR/PRR (polybasic/autoinhibitory region)] are at 229-284 and 292-340 residue. after the preparation is carried out, a protein with additional hydrogen atoms is produced, additional Gasteiger charged, without water molecules and the binding site for the ligand at the center point of the grid box with coordinates x=17.246, y=49.571, dan z=10.014.
3.2 Validation of between p47-phox and Apocynin

The result of molecular docking between macromolecule p47-phox with apocynin shows three site binding at p47-phox from the result of study are Asp 221, Arg 302, dan Arg 316 which can be seen at figure 1 and table 1.

3.3 Results of Molecular Docking of Phytochemical Compounds with p47-phox

Based on 442 Indonesian phytochemicals which registered in Indonesian herbal database, had 3D conformation, and met lipinski’s rule of five criteria. The results of the validation of herbal phytochemical compounds which had a lower average bond energy than apocynin, there are 333 phytochemical compounds. The results of visualization with PyMol resulted in 138 phytochemical compounds that have interactions with p47-phox. There are 85 phytochemical compounds that had one bond location on the p47-phox. The visualization results show that there are 60 phytochemical compounds that had two residual bonding sites on p47-phox. From the result of bond energy and bond location, the best is Morindone. The results can be seen in Figures 2 and 3. The location of the Morindone bonds at Arg 302, Arg 316, and Arg 318. These results can be seen in table 1.

3.4 Results of the Types of Phytochemical Interactions Results of Molecular Docking

The results of molecular docking showed the location of the bonds on the macromolecular residues and the bonding interactions formed by phytochemicals and p47-phox. At the residue Arg 302 binds to Morindone with hydrogen bonds. Meanwhile, the residues of Arg 316 and Arg 318 bind to Morindone with van der walls bonds.

3.5 Results of Lipinski’s Rule of Five Phytochemical Criteria

Lipinski’s Rule of Five is a method for screening compounds that can be used to form a drug design so that it is easily absorbed by the body. The results of the analysis of Lipinski’s rule of five from Morindone can be seen in table 1. As mentioned earlier, this literature review will be limited to journals published in 2010 through 2018. The time span is to see if research on the feature independence assumption on the Naïve Bayes method is still relevant. In Figure 3 it can be seen that the trend of research from 2010 to 2016 has increased, so it can be concluded that research on the assumption of attribute independence on the Naïve Bayes method is still very relevant to date.
Table 1. The results of the molecular docking test for phytochemical compounds were compared with apocynin

| Compound (ID Pubchem) | Chemical Formulas | Bond Energy (kcal/mol) | Molecular Weight (g/mol) | Log P | H-bond donor | H-bond acceptor |
|-----------------------|-------------------|-----------------------|-------------------------|-------|--------------|-----------------|
| Apocynin              | C24H20O10         | -5.5                  | 468.4                   | 1.9   | 7            | 10              |
| Morindone (442756)    | C15H10O5          | -7.7                  | 270.24                  | 3.3   | 3            | 5               |

3.6 Validation between P47-phox and Apocynin
Apocynin has been shown in vivo to inhibit NADPH oxidase activity by blocking the active site of p47-phox to bind with p22-phox. So this confirms that apocynin, although not specific, is a Nox inhibitor [9]. The result of apocynin validation with p47-phox showed the site binding in the residues of Asp 221, Arg 302, and Arg 316. Residues of Arg 302 and Arg 316 were located in the polybasix region which is the autoinhibited region of p47-phox. This result was different from the research conducted by Rastogi [15], because the location of the bonds found is the residue Pro 212, Glu 218, Asp 221, Arg 302, Arg 316, Arg 318.

3.7 Analysis of Molecular Docking Results and Interaction of Phytochemical Compounds with P47-phox
Based on 442 Indonesian phytochemicals which registered in Indonesian herbal database, had 3D conformation, and met lipinski’s rule of five criteria. The results of the validation of herbal phytochemical compounds which had a lower average bond energy than apocynin, there are 333 phytochemical compounds. The lower bond energy will make it easier for a compound to bind to other compounds and is more stable [16]. Based on the RMSD analysis, 333 phytochemical compounds are also the best results by having an RMSD of 0. A ligand with a small RMSD (<2 Å) will have the energy to form a minimal conformation so that it can better draw the original condition of the ligand [17,18].

A total of 333 phytochemical compounds were looked at the location of the bonds with p47-phox and their conformation, it produced one phytochemical compound that had the same bonding location as apocynin and had conformations that matched with apocynin. The phytochemical compound is Morindone. Morindone has a binding site on the same residue as apocynin and conforms to apocynin conformation. While the bond at Arg 302 is bonded with a hydrogen bond. Arg 316 and Arg 318 bind with van der waals bonds. Hydrogen bonding occurs when one hydrogen atom binds to the free electrons of another atom. The hydrogen bond that water molecules have 5kcal / mol while the hydrogen bonds in biological molecules are 1-3 kcal / mol [19]. Meanwhile, van der waals bonds occur when two polar and non-polar atoms are close together, resulting in temporary electron fluctuations. Van der waals bonds occur when two polar and non-polar atoms are close together, resulting in temporary electron fluctuations. Van der waals bonds have an energy of 1 kcal / mol [16]. Weak bonds such as hydrogen bonds and van der waals are needed for a drug to bind to its receptors because it can produce strong and stable bonds [20].

3.8 Analysis of Lipinski's Rule of Five Criteria for Phytochemical Compounds
Lipinski's Rule of Five criteria is a criterion for estimating the bioavailability of a drug in humans [21]. The phytochemical compound Morindone based on the screening results showed that it met the criteria of Lipinski's Rule of Five. The lower the weight of a compound, the easier the compound will be absorbed by the body [21]. The H bond donor and the H bond receptor of a compound describe the interaction of a strong compound with a solvent containing H atoms such as water, this will decrease its permeability [22]. Meanwhile, lipolificity describes a compound in a cell bilayer [22]. Recent
reviews have shown that the strength of compounds in passing through the bilayers is maximized when the lipophilicity of a compound is in a value between 1 - 3 [23].

Based on the screening results of Lipinski's Rule of Five criteria of the three compounds, the results were:

a. From the molecular weight obtained 270.24 daltons. Morindone fits the criteria because it is less than 500 daltons.
b. The number of hydrogen donors is 3. Morindone fits the criteria because it is less than.
c. The number of acceptors for hydrogen is 5. Morindone is appropriate because it is less than.
d. From the lipophilicity value, it was obtained 3,3. Morindone fits the criteria because it is less than 5.

4. Conclusion
Morindone binds to P47-phox in one residue in the SH3B region and one residue in the PBR region which is important for P47-phox phosphorylation and becomes a new candidate of NADPH oxidase inhibitor for hypertension therapy.

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