ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 2, 2015

ISSN - 0974-2441

Research Article

PHARMACOGENOMIC ANALYSIS OF SINGLE-NUCLEOTIDE POLYMORPHISMS OF ANGIOTENSIN-I CONVERTING ENZYME GENE IN STROKE

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Received: 08 January 2015, Revised and Accepted: 31 January 2015

ABSTRACT

The commonly occurring neurological disorder is stroke disease, which is a third largest killer in the world. Study of genes involved to cause stroke is one of the way to find drugs for prevention or treatment of stroke. The purpose of the present study is to develop therapeutics by giving individualized medicine based on significant single-nucleotide polymorphisms (SNPs) involved in the pathogenesis of disease. Genome-wide association approach scans the entire genome looking through thousands of genetic variants SNPs with an aim to discover novel gene associated with a specific disease. In the present study, the most intensively investigated candidate gene is angiotensin-I converting enzyme (ACE). The normal and mutated forms of ACE gene are selected, and the active site of predicted protein structure is identified where the selected ligand binds. The selected ligands of both existing and modified molecules were docked with predicted protein using AutoDock tool. The docking results were analyzed based on the maximum number of hydrogen bond interactions. The results predicted that anti-hypertensives, anticoagulants, tissue plasminogen activators (TPAs) have strong interaction with stroke genes. The docking studies showed that ACE gene strongly interacts with r-TPA and is used for treatment of strokes.

Keywords: Stroke, Single-nucleotide polymorphism, Angiotensin-I converting enzyme, Individualized medicine.

INTRODUCTION

Stroke is a common neurological disease and a leading cause of severe disability and death in developed countries [1]. According to WHO the incidence of the disease in India is around 130 per 100,000 populations every year [2,3]. Stroke's unconsciousness or impair breathing or heart function are particularly serious. Functioning problems that continue after 6 months are most likely to be permanent, although some people continue to improve slowly. People who are younger and better in general health tend to recover faster and more completely.

Genetic studies have been proposed to reveal the pathogenetic basis of stroke, which might become a therapeutic target for new drugs. Recently, genetic studies have moved to pharmacogenomics that involves a genome-wide association approach, which scans the entire genome looking through thousands of genetic variants with an aim to discover novel genes associated with a specific disease. Various types of genetic polymorphisms have been suggested to contribute to the risk of stroke [4]. There is a need to identify novel targets for preventive therapeutics [5].

Angiotensin-I converting enzyme (ACE) gene is one of the most intensely studied genes because of the key role it plays in the renin-angiotensin system. ACE catalyzes the conversion of angiotensin I to angiotensin II, a vasoactive and aldosterone-stimulating peptide and inactivates bradykinin [6]. ACE gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. The insertion deletion (I/D) polymorphism in this gene refers to an Alu repetitive sequence 287 bp long, in intron 16, resulting in three genotypes, DD and II homozygotes and ID heterozygotes. I/D polymorphism is reported to determine circulating and tissue ACE levels, such that individuals homozygous for the D allele have higher tissue and plasma ACE concentrations than heterozygotes and II homozygotes [7,8]. I/D polymorphism are associated with cardiovascular diseases as well as chronic renal diseases [9]. The gene variant in ACE gene insertion/deletion polymorphism has been identified as a potential genetic risk factor for essential hypertension [10]. I/D polymorphism analysis in the intron 16 of the ACE gene (17q23) with Alu repeats were identified [11]. In the present study, ACE gene is selected and is docked with the drugs, which are selected as ligands.

METHODS

Hardware and software

Docking calculation was calculated on Windows 7 operating system with 50 GB hard disk and 1 GB RAM and Pentium 4 processor. AutoDock tool is used for docking process and molegro molecular viewer for visualization of docked results.

Genome wide association study

Genome wide association study of stroke genes shows hundreds or thousands of genome variations (SNPs), selected from dbSNP database. The analysis shows ACE gene is significantly associated with stroke disease. The genome-wide association studies (GWAS) results with several gene variations. The gene mutations of ACE gene were screened by using the SIFT database.

Based on the probability values of ACE obtained from the GWAS, particular gene variant is selected. The gene variants which are considered to be damaging or probably damaging are removed based on this probability values. The selected gene sequence is used to predict the template using Swiss model. This template must be having 85% and above similarity to the gene. The resultant template is used to build the three-dimensional (3D) structure and further used for drug docking studies.

Protein structure prediction

The selected target protein sequence is used to predict the 3D structure using Swiss model.

Ligand preparation

Some existing anti-hypertensive agents, tissue plasminogen activators (TPA), anticoagulants and anti-platelets compounds were retrieved from PubChem compound database [12]. These existing ligand molecules are used as potential drug molecules for stroke. The formulated ligand molecules were modified based on physical and chemical properties. The training sets of lead molecules were generated through conformational search module, and further implementation has been done by Hyperchem Professional 7.0.

Molecular docking

The docking analysis of existing ligands and modified ligands was done with stroke polymorphic proteins. Docking is carried out by means

Table 1: Normal and modified anticoagulant and antihypertensive drugs (based on energy minimization and drug properties)

Drug name	Molecular weight	H-bond donor	H-bond acceptor	Rotatable bonds	LogP
Normal amlodipine (anti-hypertensive)	408.876	3	7	0	1.64
Modified amlodipine (anti-hypertensive)	524.948	4	11	12	1.64
Normal ASA (anticoagulant)	177.112	5	7	4	−4
Modified ASA (anticoagulant)	153.135	3	4	1	1.3

ASA: Acetylsalicylic acid

of the AutoDock tools v 1.5.4 and AutoDock v 4.2 programs [13]. The AutoDock 4.2 uses molecular simulation method i.e. interaction is shown by a genetic algorithm.

RESULTS AND DISCUSSION

The gene selected was ACE of GWA study with 5147 gene variants. The gene mutation screening shows 31 gene variants of SNPs within SIFT database of protein sequence NP-000780. The genome wide association study of cardiovascular disorders shows that there are many significant gene variants of ACE gene showing under the threshold for genome-wide significance of 3×10^{25}. Out of these gene variants of SNPs, rs4340 of 288BP I/D Alu repeats are observed in Indian population. To identify the nsSNP stroke genes that affect protein structure, the selected nsSNPs were analyzed for predicting possible impact of amino acids on the structure and function of protein. The protein sequence of ACE (NP-000780) has 1306 amino acids.

Modeling of protein structures

The ACE sequence of stroke gene with structurally predicted template 2c6nA chain is used as a template to predict the three dimensional structure of protein. This structure is used for molecular docking as a normal protein. The modeled protein structure is used to modify the amino acids in mutational sites. Wherever there is gene polymorphism is present in protein, that is being modified, and this protein is also used as mutated protein structures. The structures were predicted, which are shown in Fig. 1 is normal ACE and Fig. 2 mutated ACE.

Ligand selection and pharmacophore analysis

The pharmacogenomic properties of stroke inhibitory lead compounds were retrieved from Pharma GKB. The anti-hypertensive, anticoagulant, anti-platelets, statins and TPAs help for stroke inhibition. The anti-hypertensive drugs such as atenolol, amlodipine, chlorthalidone, doxazosin, perindopril, losartan, verapamil and lisinopril are effective molecules for cardiovascular and stroke diseases. The anticoagulant drugs such as acetylsalicylic acid (ASA), aspirin, clopidogril, phenprocoumon, prasugrel, and ticagrelor, finally the reference drug such as TPA is also used for stroke gene variant inhibition. The pharmacophore and pharmacokinetic properties of these compounds were studied and are listed in Tables 1 and 2. The pharmacogenomic properties within biological properties were analyzed using Hyperchem 7.5 Professional. The resultant ligand molecules were used for molecular docking.

Active site prediction

The biological property of all modeled protein structures is used to predict ligand binding site, i.e. active site amino acids using CastP. The ACE protein active site amino acids such as His390, His394, Glu418, His988, His992, Glu1016, Tyr1166 and Arg1232 are covered within active site area of 4409.3 A^3 and volume of 7809 A^3. The active site of ACE is shown in Fig. 3.

Molecular docking

The ACE protein structure of 3D model of both normal and mutated macromolecules is used for molecular docking. The anti-hypertensive, anticoagulants and TPAs used for cardiovascular and stroke disorders. The pharmacophore modeled ligand molecules of 12 compounds were selected from PubChem compound database and are used for molecular simulation and docking. The best docking result obtained is ACE with r-TPA shown in Fig. 4. Table 3 shows the interaction of ACE with r-TPA.

Table 2: TPA (based on energy minimization and drug properties)

QSAR properties	Before modeling	After modeling
Partial charges	−0.12e	0.00e
Surface grid	1116.70A	994.84
Volume	2194.32A	2100.63
Log P	−21.38	1.12
Refractivity	198.95	154.82
Polarization	90.81	58.45
Mass	942.08	898.74

TPA: Tissue plasminogen activator

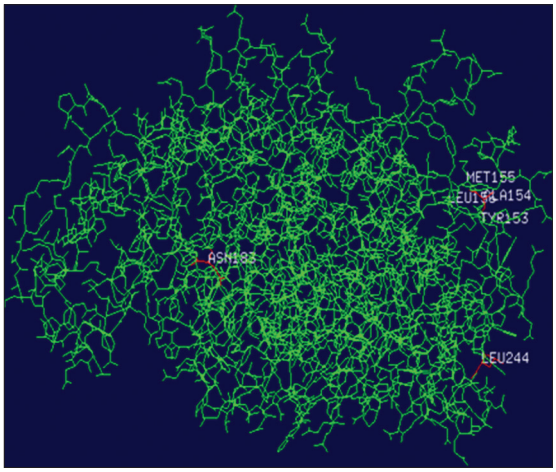

Fig. 1: Normal angiotensin-I converting enzyme protein structure

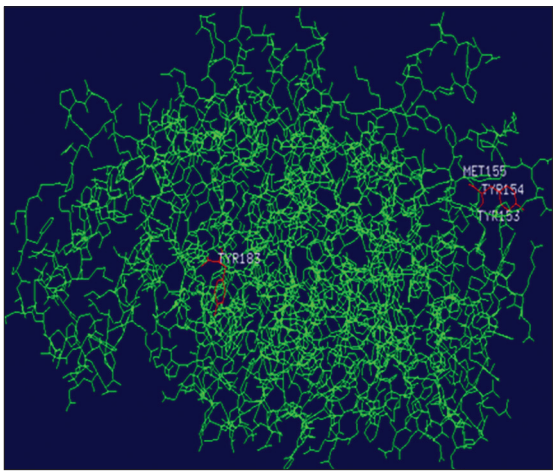

Fig. 2: Mutated angiotensin-I converting enzyme protein structure

The main findings of the present study can be summarized as follows:

The anti-hypertensives studies show nine subjects (ligands) taking strong interaction. With normal ACE protein perindopril and losartan

Table 3: Normal ACE protein interacting with modified r-TPA

Ligands	H-bonds	H-bond energy	Inhibitory constant	Internal energy	Electrostatic energy	Amino acids
TPA	9	−28.2019	210.30	−7.0815	1.43	ARG89, ARG90, THR97, SER200 SER378, SER548 TRP552

ACE: Angiotensin-I converting enzyme, r-TPA: Tissue plasminogen activator

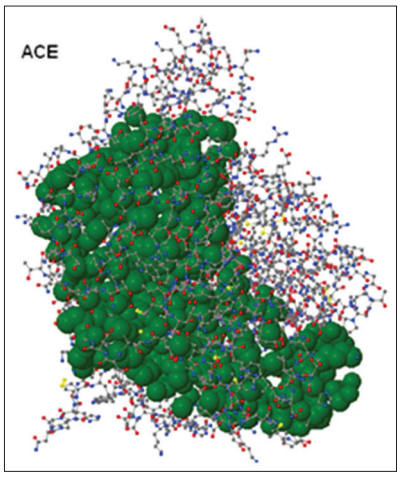

Fig. 3: Active site of angiotensin-I converting enzyme

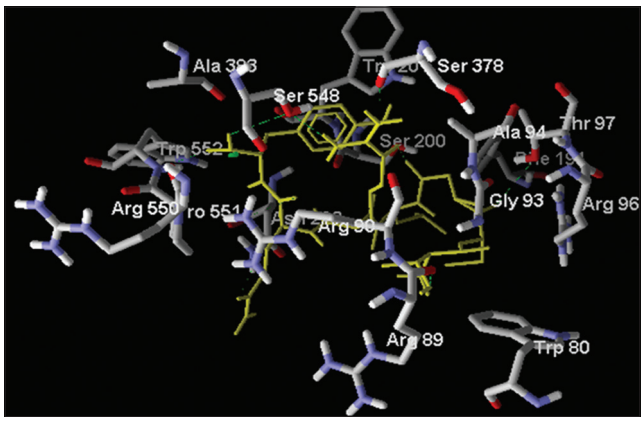

Fig. 4: Docking result of normal angiotensin-I converting enzyme protein interacting with r-tissue plasminogen activators

have strong interaction with active site amino acids. The mutated ACE protein interacts with existing anti-hypertensive drugs shows a strong interaction with amlodipine, chlorthalidone, doxazosin, perindopril, losartan and lisinopril. All these compounds are used as a potential treatment for ACE gene mutations.

The modified anti-hypertensive drug shows a strong interaction with normal and mutated protein. The ligands amlodipine, atenolol, chlorthalidone, doxazosin, lisinopril, losartan and perindopril show very strong interactions with ACE protein active site amino acids.

The category of anticoagulants shows strong interactions with ACE proteins. The interaction of existing anticoagulant ligands include ASA, aspirin, clapidogril, phenprocoumon, prasugrel and ticagrelor, these ligands shows interactions with normal protein and mutated protein and potentially used as a drug molecules to cure stroke disorder. Phenprocoumon, prasugrel and ticagrelor show very strong interaction with ACE protein and these molecules is potentially used for treatment of hypertension, and myocardial infarction and other stroke associated diseases.

The ACE gene interacts strongly with TPA and r-TPA and is used for the treatment of stroke. The mutated form of ACE also has strong interaction with both TPA and r-TPA. An r-TPA is a thrombolytic drug that inhibits thrombolysis. The ACE gene normally converts ACE-I to ACE-II using different enzymes. The mutation of ACE gene shows blood clot in nerves and cause stroke. The r-TPA drug helps to inhibit thrombolytic.

The overall results shows the active sites SER123, PRO275, HIS406, ARG413, SER420 and GLN527 amino acids strongly interacts with anti-hypertensive and anticoagulant drugs. These active sites are mainly used as potential drug targets in ACE gene.

CONCLUSION

This study predicts that pharmacogenomics of stroke as a promising approach for optimizing treatment strategies aimed at decreasing stroke incidence and recurrence. The anti-hypertensive, anticoagulants and TPAs have strong interaction with stroke genes. The ACE gene is strongly interacts with r-TPA, and anti-hypertensive drugs.

REFERENCES

1. Bonita R. Epidemiology of stroke. Lancet 1999;339:342-4.
2. Banerjee TK, Choudhary D, Das A, Sekhar A, Roy D, Sen S. Analysis of hospital-based stroke registry in a neurological centre in Kolkata. J Indian Med Assoc 2005;103(12):665-8.
3. Padma MV, Kaul S. Incidence of recurrent stroke in primary care during preventive treatment based on perindopril with or without indapamide. Neurol India 2007;55(2):141-4.
4. Munshi A, Kaul S. Stroke genetics - An overview. Neurol India 2010;58(2):181-7.
5. Kaul S, Munshi A. Genetics of ischemic stroke: Indian perspective. Neurol India 2012;60(5):498-503.
6. Erdös EG, Skidgel RA. The angiotensin I-converting enzyme. Lab Invest 1987;56:345-8.
7. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86(4):1343-6.
8. Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F. Angiotensin I-converting enzyme in human circulating mononuclear cells: Genetic polymorphism of expression in T-lymphocytes. Biochem J 1993;290 (Pt 1):33-40.
9. Hohenfellner K, Wingen AM, Nauroth O, Wühl E, Mehls O, Schaefer F. Impact of ACE I/D gene polymorphism on congenital renal malformations. Pediatr Nephrol 2001;16(4):356-61.
10. Das M, Pal S, Ghosh A. Angiotensin converting enzyme gene polymorphism (insertion/deletion) and hypertension in adult Asian Indians: A population-based study from Calcutta, India. Hum Biol 2008;80(3):303-12.
11. Srivastava K, Srivastava A, Mittal B. Angiotensin-converting enzyme insertion/deletion gene polymorphisms and risk of intracerebral hemorrhage: A meta-analysis of epidemiologic studies. DNA Cell Biol 2008;29:8.
12. Available from: http://pubchem.ncbi.nlm.nih.gov/search/search.cgi. [Last accessed on 2014 Feb].
13. Available from: http://www.scripps.edu/mb/olson/doc/autodock. [Last accessed on 2014 May].