International Journal of Research Instinct
(Peer-reviewed Journal)

ISSN ONLINE 2348-2095
Research Article

AN ANALOGUE BASED DRUG DESIGNAGAINST CRP USING COMPUTATIONAL METHODS

[1] N. RameshKannan, [2] R. Sivasubramanian and [3] N. Agnel Arul John

[1,2 &3]SRRCARB, Srimad Andavan Arts & Science College (Autonomous),Trichy– 5.
[1]rameshkannan.n@gmail.com, [2]biosiva2000@gmail.com& [3]aruljohnagnel@gmail.com

ABSTRACT

C-Reactive Protein (CRP) is the only marker of inflammation that independently predicts the risk of heart attack. CRP is phylogenetically conserved plasma protein that is present in human blood. Each anti-heart attack drugs docked with CRP at different or near active site with varied bond lengths. Based on the distance of docking region between the target and the drug molecule, the specificity and efficiency of the molecule as well as pharmacy kinetics of the drug can be determined. By using computational method, we have identified the domain region (25^{th} to 220^{th} position) and active site (Gln60, Ser62, Phe69, Asp88, Thr108, Thr116, Gly131 and Gly154) of the CRP. The 3D structure of CRP was validated by SAVS and quality factor was 76.882. The motif regions were predicted and sequence was found to be Ser23, Tyr37 and Gly131 to Val145. Seventeen major anti-heart attack drugs were chosen and were docked with the CRP's receptor and their bond lengths were calculated. Pro220, Tyr218, Lys219 and Val212 of CRP were closely bound to the drugs during docking. Among the 17 drugs, lovastatin has the lowest distance (3.63). It showed its maximum efficiency due to the high interaction with CRP. Then, the analogues of lovastatin were designed and docked with CRP and result showed Simvastatin ([(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3, 7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] (2S)-2-methylbutanoate) has a lowest distance (3.35). Then, we have suggested that the lowest distance showing drug may reduce the level of CRP and decrease the risk of heart attack.

Keywords

Drug Discovery, C Reactive Protein, Molecular Modeling, Docking, CADD

1. INTRODUCTION

The drug industry is one of the major players involved in the development of Bioinformatics. Many pharmaceutical companies have internal units conducting bioinformatics research. The objective behind this is purely commercial. The purpose is to beat the competition for finding the solution to a problem that may give their companies that crucial edge in producing the major drug.

Drug resistance has become more and more of a problem in treating heart attack in human. New drugs are needed to replace those that have been developed by creating novel analogues of established. It is likely that most anti-heart attack drug design will be based on existing drug molecule structures, but an increasing number of new drug's molecular structures may emerge from screening against the particular disease. Other importance of the analogue study is involved into the reducing the side effects in one person to person. The analogue preparation is new and important high through put technology for modern new drug discovery process of pharmacy.

C-reactive protein (CRP) is a plasma protein, member of the pentraxin family, an acute phase protein produced by the liver. It is a phylogenetically conserved plasma protein that is present in the blood[1]. The CRP gene is located on the first chromosome (1q21-q23)[2]. CRP is a 224 residue protein with a monomer molar mass of 25106 Da, and native cyclic pentamer mass of 125530[3]. CRP is one of the plasma proteins known as acute-phase proteins whose plasma concentrations increase (or decrease) by 25% or more during inflammatory disorders[4]. Since inflammation is believed to play a major role in the development of coronary artery disease, markers of inflammation have been tested in respect to heart health[5].

C-reactive protein is known as an emerging recognized marker of the potential risk of myocardial infarction and stroke[6].It possesses numerous cardiovascular effects that could result in cardiovascular disease[7]. CRP was identified for the first time involvement in early and delayed ischemic and pharmacological in an *in vivo* model of rat myocardial infarction [8] and has been identified as predictor for cardiovascular disease (CVD) for both men and women[9]. Its level was correlated with the clinical severity of CAD and with coronary events in both the acute and sub-acute phases of myocardial ischemia[10]. It is a novel and evolving biomarker for the extent and severity of cardiovascular disease and provides a useful predictive indicator for heart attack[11], diabetes, hypertension and CVD[12].

A decade ago, there was a link between anti-CMV antibodies and high CRP levels in patients with CAD was demonstrated [13]. CRP levels do not affected by lipid lowering agents, ACE inhibitors, ARBs, antidiabetic agents, antiinflammatory and antiplatelet agents, vitamin E, and beta-adrenal receptor antagonists lower serum or plasma levels of CRP, while vitamin C, oral estrogen and hydrochlorothiazide [14].

In this study, we have carried out with the aim of developing suitable drug candidates for the heart attack disease. Out of the pool compounds, Aspirin[15], Atorvastatin[16], Catoprill[17], Clopidogrel[18], Celecoxib[19], Ezetimibe[20], Fenofibrate[21], Fosinopril[22], Lovastatin[23], Olmesartan[24], Ridrogrel[25], Ramprill[26], Timolol[27], Rofecoxib[28], Quinaprill[29], Tirofiban[30] and Valsartan[31] drugs for heart attack was chosen based on their effectiveness. These drugs were docked with the receptor of CRP. The distance between drug and protein should be less than 10 angstroms.

2. MATERIALS AND METHODS

The computational methods of 3D structure building is involving with template selection, alignment with the target, building the model, validation and refinement the structure and docking with selection of suitable ligand, receptors and binding of molecules.

Template Selection

The protein sequence is retrieved from Swiss-Prot. BLAST[32] (Basic Local Alignment Search Tool) used for find an experimentally (X-ray diffraction and NMR) solved structure as a template by using Protein Data Bank (PDB), Blosum 80 as scoring algorithm matrices[33] and program blastp 2.2.19 in BLAST[34]. Then, CLUSTAL X[35] is used for find a homologous 3D structure with more than 35% identical sequence with the target sequence to do multiple sequence alignment in which The percentage of identity between the query and the template sequences are distinguished based upon their colours. NJplottree[36] is used for construct a phylogenetic tree to view the closely similar sequences among the hits which are obtained through blastp program. Bioedit[37] (developed by to Ibis Biosciences which is a sequence alignment editor and sequence analysis program) has been used for find hydrophobic and hydrophilic regions in the form of plots.

2.1 HOMOLOGY MODELLING

The raw sequence of target is fit with 3D structure of template by using DEEPVIEW V4.0[38] which is developed by Nicolas Guex , AlexandreDiemand , Manuel C. Peitsch , &TorstenSchwed in Swiss Institute of Bioinformatics. The structurally aligned backbone molecules submitted to SWISS-MODEL[39], an automated Comparative Modelling Server, to perform the molecular dynamics and energy minimization to the aligned model. This protein model is evaluated by using SAVS.

2.2 ACTIVE SITE

To find the active sites of modeled target protein, CASTp[40] server is used. Then, Jmol, a free and open source molecule viewer, is used for view the pocket information from CASTp.

2.3 MOTIF REGION

The motif region of the protein can be predicated by using the tool motif scanner[41]. The Fasta format of the target protein is submitted in motif scanner. The motif region of amino acid is noted and its structure is visualized in Deep View.

2.4 DOCKING

Docking[42] is a research technique for predicting whether one molecule will bind to another, usually a protein. Protein-protein, Protein-DNA and protein-ligand docking predications are all performed, though the techniques employed in each area are highly various. Protein-ligand docking is done by modelling[43]. The interaction between protein and ligand; if the geometry of the pair is complementary and involves favourable biochemical interactions, the ligand will potentially bind the protein *in vitro* or *in vivo*. The Docking process can be done by using the software Hex docking software[44].

3. RESULT AND DISCUSSION

This study was carried out with the novel aim of developing suitable drug candidates for the heart attack disease. CRP is the inflammatory protein responsible for heart attack[45]. Molecular modelling is the method of choice when there is a close homology between the sequence of the target protein and the template[46]. Comparative modeling frequently provides a useful model of 3D structure[47]. Swiss-PDB viewer helps to resolve the problem effectively.

In this study, the 3D model of unmodelled chain A of CRP protein was built from 3D modelled protein (Fig. 1) which is identical with target sequences and 3 D structure of target (Fig. 2) was generated by using Deepview. The domain region of the CRP was predicted by using a Pfam tools. It found to be start from 25th position of sequence and end from 220th position of sequence. The active site of the CRP was predicted by using the tool CASTp which identified the amino acid residues present at the active site of CRP. They were Gln60, Ser62, Pha69, Asp88, Thr108, Thr116, Gly131 and Gly154 (Fig. 3). The 3D validation of the predicted homology structure of CRP (Fig 2) can be assessed by SAVS. The quality factor of protein was found to be 76.882. The motif region of the sequence found to be Ser23 to Tyr37, Gly131 to Val145 (Fig. 4).

The drug candidates chosen for this study is commercially marketed drugs for heart attack in order to reduce the level of CRP. Huddles of drugs were identified through Drug Bank[48]. Out of the pool compounds Aspirin[15], Atorvastatin[16], Catoprill[17], Clopidogrel[18], Celecoxib[19], Ezetimibe[20], Fenofibrate[21], Fosinopril[22], Lovastatin[23], Olmesartan[24], Ridrogrel[25], Ramprill[26], Timolol[27], Rofecoxib[28], Quinaprill[29], Tirofiban[30] and Valsartan[31] drugs for heart attack was chosen based

on their effectiveness. These drugs were docked with the receptor and their bond lengths were tabulated (Table 1). The distance between drug and protein should be less than 10 angstroms. Some of the few amino acids, which are closely bound to drug molecules were Pro220, Tyr218, Lys219, and Val 212. The distance of Lovastatin (Fig. 5) showed its maximum efficiency (Fig. 6). It may be due to more interactions with the target CRP. So the analogue structure of Lovastatin was created and docked with the CRP.

Analogues are compounds in which one or more individual atoms have been replaced, either with a different atom or with a different functional group. Analogues structure of Lovastatin was found to be

1. Simvastatin ([(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3, 7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] (2S)-2-methylbutanoate)
2. Pravastatin (3R,5R)-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(2S)-2-methylbutanoyl]oxy-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid
3. Fluvastatin ((E,3S,5R)-7-[3-(4-fluorophenyl)-1-propan-2-ylindol-2-yl]-3,5-dihydroxyhept-6-enoic acid)

These three designed drugs were docked with CRP to reduce the level of protein. These result suggest that Simvastatin ([(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3, 7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] (2S)-2-methylbutanoate) (Table 2) Fig. 7 and 8) showed the distance of 3.35 angstroms which was very affinity than others. So this designed drug may reduce the level of CRP and decreased the risk of heart attack.

4. CONCLUSION

The study describes the computational analysis of potential interactions of this molecule with the analogues of Lovastatin. This CRP molecule has been identified as a potential heart diseases' marker, and the interactions observed with analogues of Lovastatin suggested that this compound may have potential as a lead for future drug design for heart diseases. Further investigations are required for a better understanding of how CRP and Lovastatin derivatives bind at their site. This work provides comprehensive insight and understanding for the molecular interaction of CRP with other antiheart drugs especially with Lovastatin.

REFERENCES

1. Oliveira E.B., Gotschlich E.C., Liu T.-Y, Primary structure of human C-reactive protein, J. Biol. Chem,**254**, 489-502, 1979.

2. Whitehead A.S., Bruns G.A.P., Markham A.F., Colten H.R., Woods D.E., Isolation of human C-reactive protein complementary DNA and localization of the gene to chromosome 1,Science,**221**, 69-71, 1983.
3. Murzin AG, Brenner SE, Hubbard T, Chothia C., SCOP: a structural classification of proteins database for the investigation of sequences and structures, J Mol Biol., **247**, 536-40, 1995.
4. Clyne, Brian; Jonathan S. Olshaker, The C-reactive protein, Journal of Emergency Medicine,**17**, 1019–1025, 1999.
5. Lloyd-Jones DM, Liu K, Tian L, Greenland P., Narrative review: assessment of C-reactive protein in risk prediction for cardiovascular disease, Ann Intern Med, **145**, 35–42, 2006.
6. Sung KC., Seasonal variation of C-reactive protein in apparently healthy Koreans, Int J Cardiol., **107**, 338-42, 2006.
7. Umesh N. Khot, MD; Monica B. Khot, MD; Christopher T. Bajzer, MD; Shelly K. Sapp, MS; E. Magnus Ohman, MD; Sorin J. Brener, MD; Stephen G. Ellis, MD; A. Michael Lincoff, MD; Eric J. Topol, MD, Prevalence of Conventional Risk Factors in Patients With Coronary Heart Disease, JAMA, **290**, 898-904, 2003.
8. ValtchanovaMatchouganskaM. Gondwe and A. Nadar , The role of C-reactive protein in ischemia/reperfusion injury and preconditioning in a rat model of myocardial infarction, Life Sciences, **75**, 901-910, 2004.
9. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH, Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men, N Engl J Med., **336**, 973 -979, 1997.
10. Morrow DA, Cannon CP, Jesse RL, *et al*. National Academy of Clinical Biochemistry. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes, ClinChem, **53**, 552–74, 2007.
11. David A. Morrow MD, Nader Rifai, Elliott M. Antman MD Debra L. Weiner MD, Carolyn H. McCabe BS, Christopher P. Cannon MD, and Eugene Braunwald MD, C-Reactive Protein Is a Potent Predictor of Mortality Independently of and in Combination With Troponin T in Acute Coronary Syndromes: A TIMI 11A Substudy, Journal of the American College of Cardiology, **31**, 1460-1465, 1998.
12. John Danesh; Wheeler, JG; Hirschfield, GM; Eda, S; Eiriksdottir, G; Rumley, A; Lowe, GD; Pepys, MB et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease, New England Journal of Medicine, **350**, 1387–1397, 2004.

13. Zhu J, Quyyumi AA, Norman JE, et al. Cytomegalovirus in the pathogenesis of atherosclerosis: the role of inflammation as reflected by elevated C-reactive protein levels. J Am CollCardiol.,**34**, 1738–1743, 1999.
14. Kailash Prasad, C-Reactive Protein (CRP)-Lowering Agents, Cardiovascular Drug Reviews, **24**, 33-50, 2006.
15. Schwartz KA, Aspirin resistance: a review of diagnostic methodology, mechanisms, and clinical utility. AdvClin Chem.,**42**, 81-110, 2006.
16. Rouleau J., Improved outcome after acute coronary syndromes with an intensive versus standard lipid-lowering regimen: results from the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial., Am J Med.,**118**, 28-35, 2005.
17. Atkinson AB, Robertson JIS., Captopril in the treatment of hypertension and cardiac failure,Lancet,**2**, 836–9, 1979.
18. Dorsam RT, Murugappan S, Ding Z, Kunapuli SP, Clopidogrel: Interactions with the P2Y12 Receptor and Clinical Relevance, Hematology,**8**, 359-65, 2003.
19. Jones DA, Carlton DP, McIntyre TM, Zimmerman GA, Prescott SM., Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines, J Biol Chem., **268**, 9049-9054, 1993.
20. Altmann SW, Davis HR Jr, Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, Graziano MP, Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption, Science,**303**, 1201-4, 2004.
21. Bouly M, Masson D, Gross B, Jiang XC, Fievet C, Castro G, Tall AR, Fruchart JC, Staels B, Lagrost L, Luc G, Induction of the phospholipid transfer protein gene accounts for the high density lipoprotein enlargement in mice treated with fenofibrate, J Biol Chem.,**276**, 25841-7, 2001.
22. Kowala MC, Grove RI, Aberg G, Inhibitors of angiotensin converting enzyme decrease early atherosclerosis in hyperlipidemic hamsters. Fosinopril reduces plasma cholesterol and captopril inhibits macrophage-foam cell accumulation independently of blood pressure and plasma lipids,Atherosclerosis., **108**, 61-72, 1994.
23. Bobek P, Ozdin L, Galbavy S., Dose- and time-dependent hypocholesterolemic effect of oyster mushroom (Pleurotusostreatus) in rats,Nutrition,**14**, 2-286, 1998.

24. Mire DE, Silfani TN, Pugsley MK, A review of the structural and functional features of olmesartanmedoxomil, an angiotensin receptor blocker, J CardiovascPharmacol.**46**, 585-93, 2005.
25. No authors list, Randomized trial of ridogrel, a combined thromboxane A2 synthase inhibitor and thromboxane A2/prostaglandin endoperoxide receptor antagonist, versus aspirin as adjunct to thrombolysis in patients with acute myocardial infarction. The Ridogrel Versus Aspirin Patency Trial (RAPT), Circulation, **89**, 588-95, 1994.
26. Frampton JE, Peters DH, Ramipril. An updated review of its therapeutic use in essential hypertension and heart failure.,Drugs, **49**, 440–66, 1995.
27. Borjesson M, Magnusson Y, Hjalmarson A, Andersson B, A novel polymorphism in the gene coding for the beta(1)-adrenergic receptor associated with survival in patients with heart failure., Eur Heart J.,**21**, 1853-8, 2000.
28. Fitzgerald GA, Coxibs and cardiovascular disease.,N Engl J Med., **351**, 1709-11, 2004.
29. Khan BV, Sola S, Lauten WB, Natarajan R, Hooper WC, Menon RG, Lerakis S, Helmy T, Quinapril, an ACE inhibitor, reduces markers of oxidative stress in the metabolic syndrome.,Diabetes Care., **27**, 1712-5, 2004.
30. Wilcox DA, Paddock CM, Lyman S, Gill JC, Newman PJ,Glanzmannthrombasthenia resulting from a single amino acid substitution between the second and third calcium-binding domains of GPIIb. Role of the GPIIb amino terminus in integrin subunit association.,J Clin Invest., **95**, 1553-60, 1995.
31. Azizi M, Menard J, Bissery A, Guyenne TT, Bura-Riviere A, Vaidyanathan S, Camisasca RP, Pharmacologic demonstration of the synergistic effects of a combination of the renin inhibitor aliskiren and the AT1 receptor antagonist valsartan on the angiotensin II-renin feedback interruption., J Am SocNephrol., **15**, 3126-33, 2004.
32. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J., Basic local alignment search tool, J. Mol. Biol., **215**, 403-410, 1990.
33. Henikoff, S., Amino Acid Substitution Matrices from Protein Blocks, PNAS, **89**, 10915–10919, 1992.
34. Stephen, F. A., Thomas, L. M., Alejandro, A. S., Jinghui, Z., Zheng, Z., Webb, M. and David, J. L., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res., **25**, 3389-3402, 1997.

35. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G., TheClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, **24**, 4876-4882, 1997.
36. Perrière, G. and Gouy, M., An on-line retrieval system for biological sequence banks, Biochimie, **78**, 364-36, 1996.
37. Suhanya, R., Protein structure prediction of PINK1 in Parkinson's Disease. Advanced BioTech, **5**, 19-21, 2006.
38. Guex, N., Diemand, A. and Peitsch, M.C., Protein modelling for all.,TiBS, **24**, 364-367, 1999.
39. Arnold, K., Bordoli, L., Kopp, J. and Schwede, T., The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling.,Bioinformatics,**22**, 195-201, 2006.
40. Joe, D., Zheng, O., Jeffery, T., Andrew, B., Yaron T. and Jie L., CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated resiudes, Nucleic Acid Research, **34**, W116-W118, 2006.
41. Obenauer JC, Cantley LC, Yaffe MB., Scansite 2.0: Proteome-wide prediction of cell signaling interactions using short sequence motifs., Nucleic Acids Res. **31**, 3635-41, 2003.
42. Lengauer T, Rarey M, Computational methods for biomolecular docking, Curr. Opin. Struct. Biol., **6**, 402–6, 1996.
43. Morris RJ, Najmanovich RJ, Kahraman A, Thornton JM, Real spherical harmonic expansion coefficients as 3D shape descriptors for protein binding pocket and ligand comparisons, Bioinformatics, **21**, 2347–55, 2005.
44. Dave W. Ritchie, Hex 5.1 user manual: Protein docking using spherical polar fourier correlations, 2008.
45. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG, Genetically elevated C-reactive protein and ischemic vascular disease, N Engl J Med, **359**, 1897–908, 2008.
46. Ginalski K. Comparative modeling for protein structure prediction. CurrOpinStruct Biol., **16**, 172-7, 2006.
47. John B, Sali A., Comparative protein structure modeling by iterative alignment, model building and model assessment.,Nucleic Acids Res.,**31**, 3982-92, 2003.
48. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M., DrugBank: a knowledgebase for drugs, drug actions and drug targets, Nucleic Acids Res.,**36**, D901-6, 2008