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RESEARCH ARTICLE

Computational screening and *in silico* docking analysis of non-selective beta-blockers over beta-3 adrenergic receptors

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ABSTRACT

Background: Beta-blockers with additional beneficial activity such as beta-2 agonist and alpha-1 blocking activity are developed and available as the third-generation beta-blockers for effective management of hypertension with comorbid conditions. Hypertension associated with obesity is one such common condition seen among majority of population. **Aims and Objectives:** This study attempts to screen the existing beta-blockers currently in use for hypertension for additional beta-3 agonist activity by *in silico* prediction methods. **Materials and Methods:** The approved non-selective beta-blockers, carvedilol, celiprolol, nebivolol, nadolol, carteolol, pindolol, propranolol, timolol, oxprenolol, sotalol, penbutolol, and labetalol, are selected to screen by three-dimensional (3D) quantitative structure–activity relationship analysis, molecular docking and dynamics were carried out in CentOS Linux platform version 5.0 installed in HPZ 800 workstation using Schrodinger LLC, New York. **Results:** Among screened 12 non-selective beta-blockers, carvedilol has high free binding scores of −63.186 followed by celiprolol with −53.225 and nebivolol with −53.054. **Conclusion:** The current research by *in silic*o screening of 12 3D chemical structures of non-selective beta-blockers that effectively docked over beta 3 receptors are Carvedilol, celiprolol and nebivilol. Further studies can be carried on beta 3 cell lines followed by obesity animal models to validate our study finding.

KEY WORDS: *In silico*; Carvedilol; Celiprolol; Nebivolol; Receptors

INTRODUCTION

Beta-blockers have come a long way in the treatment of arterial hypertension with three generations of continuous upgrades in search of ideal beta-blocker with minimal side effect and additional activity to combat comorbid conditions. The third-generation beta-blockers are now most preferred drug for the treatment of hypertension having few side effects compared to older generation and additional pharmacological activity, celiprolol apart from beta-1 blocking activity has beta-2 agonist and nitric oxide production, hence, considered safer to use in asthmatics.[1]

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The beta-blockers are still avoided in diabetes because they mask initial symptoms of hypoglycemia as drug interaction and hence like celiprolol, there is still search of better beta-blocker to combat comorbid disease in hypertension such as diabetes and obesity. The discovery of beta-3 receptors and specific beta-3 receptors agonist ligands BRL 26830 A, BRL 33725 A, and BRL 35135 A has published results of tremendous antiobesity action on severe obese diabetic mice.[2] This understanding provides an opportunity to search

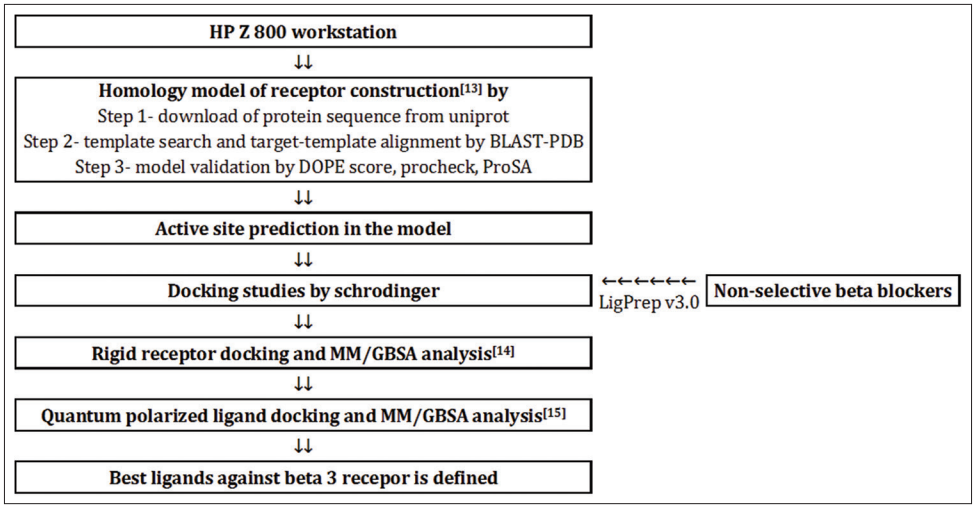

Figure 1: Multiple sequence alignment of Human ADRB3 with Turkey ADR1B (81 % Query Coverage, 51% Identity) Alignment of amino acid sequence of human beta 3 receptor. Total amino acids in the sequence are 408. The highlighted boxes indicate active sites at 117-aspartic acid and 122-threonine.

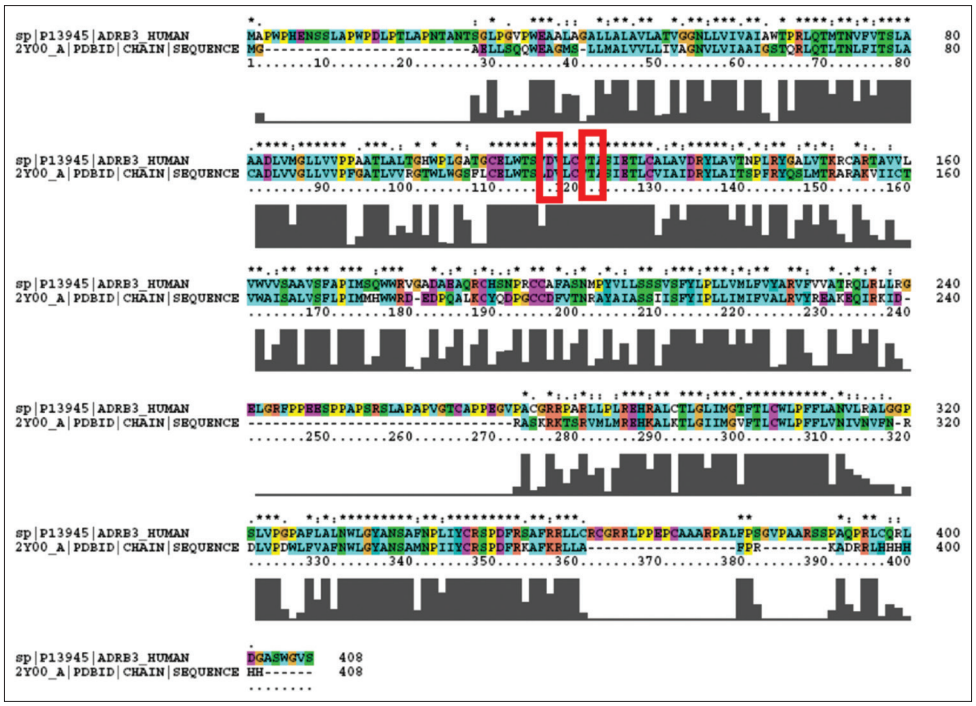

Figure 2: Human ABRB3 receptor sequence. Multiple sequence alignment of human ADR3B with Turkey ADR1B (81% query coverage, 51% identity) alignment of amino acid sequence of human beta-3 receptor. Total amino acids in the sequence are 408. The highlighted boxes indicate active sites at 117 – aspartic acid and 122 – threonine

and analyze the possibility of approved non-specific beta-blockers docking activity on beta-3 adrenergic receptor for better therapeutic profile on obesity and diabetes by computational analysis.

In silico computer simulation is considered as one of the most efficient ways to achieve 3Rs (reduction, refinement, and replacement) of animal research.[3] Computers can often predict the toxicity of chemicals, including their potential to cause cancer or birth defects, based on their molecular structure. Computer simulation can also predict the metabolism and distribution of chemicals in human tissues.[4] There are number of methods developed and validated among them QSAR models developed by the FDA with proprietary databases.[5] *In silico* computer simulation of research can provide predictive results of numerous compounds of more complex phenomena for which limited or no data available, thereby a lead compound can more easily selected for further research. Hence, the current study is a trial and error attempt in search of non-specific beta-blocker with beta-3 docking activity by computer simulation and an attempt to optimize the lead compound for better therapeutic activity.

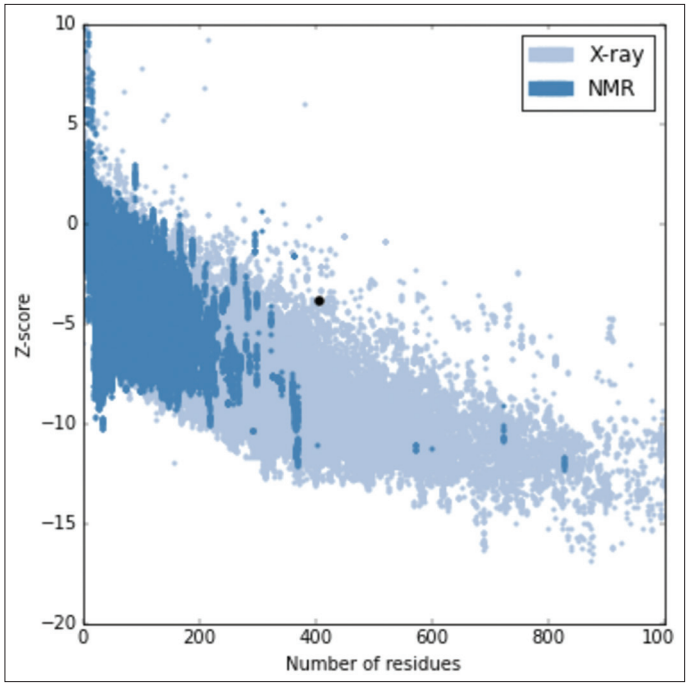

Figure 3: Z-score of modeled receptor. The Z-score graph indicates overall model quality. Above figure indicates the Z-score value of beta-3 homology model which is within the range of scores typically found for native proteins of similar size

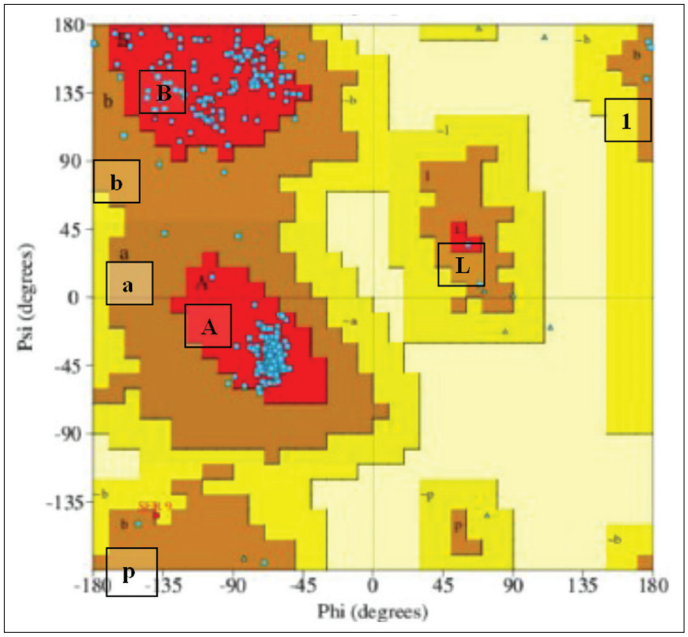

Figure 4: Ramachandran plot of modeled human ADRB3. The Ramachandran plot statistics – Model validation highlighted 94.2% (341) residues in most favored regions (A, B, L), 4.7% residues in additional allowed regions, 0.8%[3] in generously allowed regions, and 0.3%[1] residues in disallowed regions. The Ramachandran plot is used for model validation. The above beta-3 homology model plot shows most of the confirmations in favorable regions (A, B, L). Numbers of glycine residues in the plot are more which has free ends so it allows more flexibility to the model. All these show the high validity of homology model

Objectives

The objectives of the study were as follows:

1. To screen for non-selective beta-blockers with beta receptor docking activity by computer simulation
2. To identify lead compound structure *in silico* with better beta-3 docking activity.

MATERIALS AND METHODS

Hardware and Software

Three-dimensional (3D) quantitative structure–activity relationship analysis, molecular docking and dynamics of non-specific beta-blockers were carried out in CentOS Linux platform version 5.0 installed in HPZ 800 workstation using Schrodinger LLC, New York, 2014.[6]

Data Set

Sequence of adrenergic beta 3 receptor of homo sapiens (ABRB3) is downloaded from uniprot, freely accessible database of protein sequence and functional information. In a single search, Protein Data Bank (PDB) combines the protein sequence and gives the chemical structure in two dimensional and 3D pattern. The 3D chemical structure of non-specific beta-blockers is taken from accredited source PubChem. Ligprep v3.0 is used for cleaning and minimization of the energies of the structures.[7]

Virtual Screening and Docking [Figure 1]

Homology model of receptor is constructed for better activity. Initially, the protein sequence of ABRB3 is downloaded from UniProt and it was uploaded into Basic Local Alignment Search Tool-PDB which in single search makes template search, target-template alignment, and finally constructs the 3D model of receptor. This model is validated by

a. Discrete optimized protein energy score – to assess the quality of a structure model as a whole and also help in spot the problematic regions in model
b. PROCHECK – to check the sterochemical quality of the constructed model
c. ProSA – To calculate overall score of the model. It validates that the model by Z-score is to check whether the Z-score of input structure is within the range of scores typically found for native proteins of similar size. The other one is plot of residue scores which shows local model quality by plotting energies as a function of amino acid sequence position i, the positive value in graph indicates problematic regions.

Finally, homology model was constructed and then active sites in the model are predicted. The non-selective beta-blockers downloaded from PubChem are validated by LigPrep v3.0.

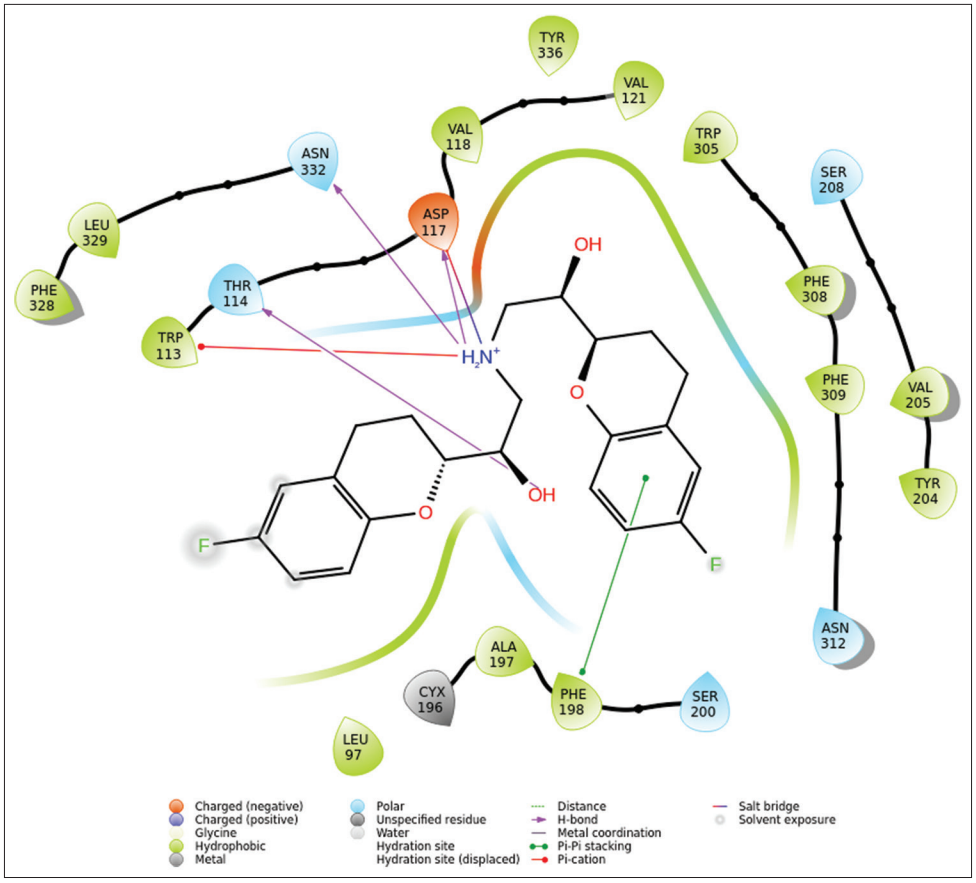

Figure 5: Docking complex of nebivolol on human ADRB3 receptor. The figure shows predicted docking site of nebivolol on human ADRB3 receptor

Both receptor model and the non-selective beta-blockers are challenged in *in silico* environment using Schrodinger software; then, there is a rigid receptor docking, followed by quantum polarized ligand docking which is a novel research solution that combines the power of glide with accuracy of Q site. Finally, docking scores of receptor-ligand complexes are obtained. The project was initiated after taking approval from the institutional ethics committee on May 4, 2017. The research is done in the department of bioinformatics and department of pharmacology, for 15 days in the month of June 2017 followed by analysis and interpretation of results.

RESULTS

The findings of the present study are depicted in Table 1 and Figures 2-5.

Free binding energy scores provide docking energy complex. Among screened 12 non-selective beta-blockers, carvedilol has high free binding scores of −63.186 followed by celiprolol with −53.225 and nebivolol with −53.054

DISCUSSION

The result of alignment from amino acid multiple sequence alignment of human adrenergic beta-3 (ADRB3) with Turkey ADRB1 is in sync. The highlighted red color boxes in the sequence [Figure 2] indicated the favorable regions in the receptor, 117 is aspartic acid and 122 is threonine, the total amino acid sequence in the protein is 408, the mass of the protein is 43,519 daltons. The Z-score of constructed homology model [Figure 3] is within the range of similar size protein Z-scores, 3D constructed model shows most of the confirmations in favorable quadrants. Results from

Table 1: Free binding energy scores of molecule docking complexes

Non-selective beta-blockers	Free binding scores
Carvedilol	−63.186
Celiprolol	−53.225
Nebivolol	−53.054
Nadolol	−50.309
Carteolol	−47.251
Pindolol	−44.941
Propranolol	−43.858
Timolol	−42.692
Oxprenolol	−39.790
Sotalol	−36.394
Penbutolol	−33.569
Labetalol	−19.239

Ramachandran plot to assess the quality of 3D structure of protein revealed that the 3D constructed model shows most of the confirmations in favorable quadrants.

The validated 3D model of beta-3 protein structure is challenged with the 12 Food and Drug Administration approved beta-blockers, carvedilol, celiprolol, nebivolol, nadolol, carteolol, pindolol, propranolol, timolol, oxprenolol, sotalol, penbutolol, and labetalol. The results of free binding energies of molecule docking complexes are listed in priority order, Table 1. Among the screened non-selective beta-blockers, the score of top compounds with least binding energy and higher stability is carvedilol −63.186, celiprolol −53.225, and the nebivolol −53.054, this shows affinity of these compounds toward beta-3 receptors. Finally, from the binding interaction studies on beta-3 receptors, the results predicted that nebivolol had established docking over beta-3 receptor by hydrogen bonding with the active site compound being aspartic acid at 117 [Figure 5].

The alignment in sequence of amino acid resulted as a source for homology model in construction of beta-3 receptor. The graph of Z-score under ProSA model is utilized for validation which indicated overall model quality. It allowed us to check whether the Z-score of input structure is within the range of scores typically found for native proteins of similar size and the constructed model is confirmed to be valid to continue the study. Further from Ramachandran plot, the torsion angles described rotation of the polypeptide backbone around bonds between N-C-alpha (phi) and C-alpha-C (psi), these angles provide flexibility by analyzing the quality of 3D structure of protein. The presence of glycine residues in the model has given flexibility to the structure, so the model is stable and more valid to challenge with test molecules. Although carvedilol, celiprolol had higher stability than nebivolol, nebivolol was preferred for further analysis based on earlier human studies, which has already reported weight loss.[8] In comparison of results from the study of Costanzi and Vilar, 2012, nebivolol had least binding energy of −10.751 compared to other 60 ligands, which was similar to the results of the present study.[9]

The strength and advantages of the present *in silico* study are to avoid harm to the animals and humans, cost effective, and reduce the time consumption to screen the compounds effectively with prediction of lead compound with minimal failure rate during preclinical animal experiments. Similarly, *in silico* studies cannot replace *in vitro*/*vivo* or whole animals in establishing pharmacodynamics and interactions.

CONCLUSION

The *in silico* screening of 12 3D chemical structures of non-selective beta-blockers by Schrodinger software has predicted that all the 12 compounds successfully docked on beta-3 receptor with various binding energies. From the free binding energy score, the effectively docked compounds are carvedilol, celiprolol, and nebivolol which would be ideal lead molecule candidates for further research. Further studies can be carried on beta-3 cell lines followed by obesity animal models to validate our study findings. A reduced parasympathetic and elevated sympathetic activity was observed in middle-aged obese males in the resting state,[10] a significantly higher resting heart rate points toward an altered autonomic balance in obese young adults,[11] and obesity and hypertension will have an adverse impact on pulmonary functions.[12] Hence, nebivolol if proved to have positive intrinsic activity, it may have potential to be optimized and developed as new indication as preferred drug for hypertensive patients with diabetes and obesity.

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