ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 3, pp.063-066, April, 2010

RESEARCH ARTICLE

MACROMOLECULAR INTERACTION AND SIMULATION STUDIES ON DUCHENE MUSCULAR DYSTROPHY

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ARTICLE INFO

Article History:
Received 13th February, 2010
Received in revised from
17th March, 2010
Accepted 27th March, 2010
Published online 7th April, 2010

Key words:
Autodock,
Duchenne,
Muscular dystrophy,
Gromacs,
Molecular modelling,

ABSTRACT

Muscular dystrophy (MD) is a group of rare genetic disorder that weakens the muscles that helps in the body movement. Although all MD are genetic disorders, the types of inheritance vary, and Duchenne muscular dystrophy arises from new mutations. The gene for DMD, found on the X chromosome, encodes a large protein—dystrophin. HDAC also define a common target for independent pharmacological interventions in the treatment of Duchenne muscular dystrophy. Dystrophin protein is modeled using the Modeller9v1 and the modeled protein is simulated for molecular dynamics studies using GROMACS. Trichostatin A is an example of a HDAC inhibitor which is being studied as a breast cancer therapy drug and has been studied in both the mdx mouse model (Duchenne) and sarcoglycan deficient mouse model (LGMD). From the binding database 175 hits were found for the HDAC1 inhibitor. These 175 compounds are then screened for the best activity against dystrophin. The detailed docking analysis of the complex structures and on their interaction energies derived by the docking study before and after the simulation at 310.15 K provided a reasonable basis for the inhibition potency of the inhibitors against dystrophin. By macro molecular interaction it is found that Benzamidine have higher affinity value compared to the commercial available drug TrichostatinA.

INTRODUCTION

Muscular dystrophy (MD) refers to a group of more than 30 inherited diseases that cause muscle weakness and muscle loss. Some forms of MD appear in infancy or childhood, while others may not appear until middle age or later. All forms of MD grow worse as the person's muscle gets weaker and many people eventually lose the ability to walk. MD is a sex-linked recessive disease. It typically passes from a mother (who has no symptoms) to son. Since MD is genetic, people are born with the problem. There are nine major types of MD affecting people of all ages. The two most common types of MD affect children are Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Duchenne Muscular Dystrophy (DMD) is characterized by rapid progression of muscle degeneration, eventually leading to loss of ambulation and death. The disorder is caused by a mutation in the gene DMD. Drugs used to treat DMD are Angiotrofin, Novo-Diltiazem-CD, Etc; No treatment can stop the progressive muscle impairment of muscular dystrophy (Mariz Vainzof *et al.,* 1993).

Normal function of the DMD gene to provide instructions for making a protein called dystrophin, is located chiefly in muscles used for movement of skeletal muscles and the muscles of the heart or cardiac muscles (Cziner and Levin, 1993). Histone Deacetylase inhibitors (HDAC) works by adding an acetyl group to histones. Histones are proteins that are associated with DNA and regulate gene activity. TrichostatinA is an example of a HDAC inhibitor which is being studied both as a breast cancer therapy drug and has been studied in both the mdx mouse model (Duchenne) and sarcoglycan deficient mouse model (LGMD) (Claudia Colussia *et al.,* 2006).

Drug discovery is the process of discovering and designing drugs, which includes target identification, target validation, lead identification, lead optimization and introduction of new drugs to the public. This process is very important, involving analyzing the causes of the disease and finding ways to tackle them. The new chemical entities (NCEs) provide insights into molecular recognition and also serve as leads for designing future new drugs (Liu *et al.,* 2008). Many effective drugs act via modulation of multiple proteins rather than single targets (Chen, 2008; Hopkins, 2008). Docking, in the field of molecular modeling, is a method, which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex, strength and type of signal, produced. In this the docking was done using Autodock (Garrett Morris *et al.,* 1999).

The motion of the atoms was understood by Molecular Simulation, in which atoms and molecules are allowed to

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interact for a period of time by approximations of known physics, giving a view of the motion of the atoms. In this molecular simulation was done using GROMACS (Groningen Machine for Chemical Simulations).

MATERIALS AND METHODS

The homology modeling of dystrophin was modeled using Modeller 9v1, which is used for homology or comparative modeling of protein three-dimensional structures (Marti-renom *et al.,* 2000). It implements comparative protein structure modeling by satisfaction of spatial restraints and can perform many additional tasks (Sali *et al.,* 1993).

The critical first step in homology modeling is the identification of the best template structure, the simplest method of template identification relies on serial pair wise sequence alignments aided by database search techniques such as FASTA and BLAST (Guillaume launay *et al.,* 2008). Blast search was implied, which gave the PDB ID: 1EG3, Chain A, Structure containing fragment of dystrophin in complex with beta-dystroglycan, as the most appropriate template with of 78% identity with human dystrophin protein.

The Modeled Structure is validated using the Procheck server where the stereo chemical quality of the modeled protein structure is analyzed (Laskowski *et al.,* 1993). The modeled protein dystrophin was found to have 95.1% all the compounds in the most favored region showing it as a good model. The stereo chemical validation of model structures of proteins is an important part of the comparative molecular modeling process (Morris *et al.,* 1992). The CE (Combinatorial Extension) Algorithm was employed for calculating the pair wise structure alignment of the template and the modeled protein. The Modeled structure of dystrophin was then docked with Histone Deacetylase inhibitors (HDAC) the only steroid proteins available for the treatment of dystrophin (Ilya *et al.,* 1998). HDAC proteins which satisfies Lipinski rule of five were analyzed and 36 compounds are found to satisfy. Other compounds were screened by virtual screening process and 16 compounds were selected for docking. The screened active compounds were further analyzed by docking and simulation studies. Docking was done using Autodock 4.2, (Garrett.M.Morris *et al.,* 1999). Understanding the ruling principles whereby protein receptors recognize, interact, and associate with molecular substrates and inhibitors, is of paramount importance in drug discovery efforts.

Protein-ligand docking aims to predict and rank the structure(s) arising from the association between a given ligand and a target protein of known 3D structure (Sérgio Filipe Sousa *et al.,* 2006), here molecular simulation is done using. GROMACS, it is used in the simulation of protein folding. After the docking process the resulting complexes was viewed in Accelrys Discovery Studio Visualizer, which provides functionality for visualizing, analyzing, and sharing biological and chemical data. The detailed docking analysis of the complex structures and on their interaction energies derived by the docking study before and after the simulation at 310.15 K provided a reasonable basis for the inhibition potency of the inhibitors against dystrophin.

RESULTS

The structure of dystrophin modeled with Modeller9v1 with 1EG3 as template. The quality of the modeled protein was evaluated using Procheck suite. On the analysis of the Ramachandran plot it was observed that 95.1% residues were present in the most favored regions. From CE, the Structural alignment of the modeled protein and the template was found to have RMSD value of 0.3A°

Molecular dynamics study of dystrophin at 310.15 K for 10.ps using GROMACS, suggests that the energy level of the simulated structure has minimized from the initial structure. No major changes were obtained in the domain region. The initial potential energy was found to be -3.50692e+004 and the energy after minimization was -5.801806+004. The position restraining requires pr.mdp file which contains the parameters required for running the position restraint simulation. An .xvg file was created which had the energy changes for 10 picoseconds. The energy plot shows that there was a decrease in energy from the initial structure to the simulated structure

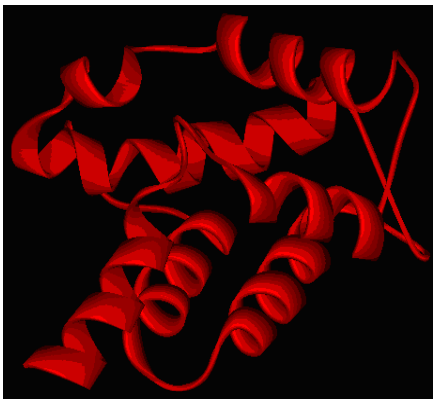

Fig.1. Structure of the Modeled protein - Dystrophin

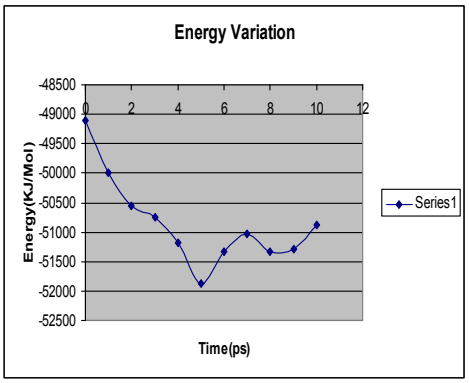

Fig.2. Energy variation Plot

Macromolecular interactions between the inhibitors and the protein was performed using Lamarckian genetic algorithm by Autodock and the energy values were tabulated for 16 HDAC inhibitors which were docked with the protein dystrophin, as shown above. ADME means absorption, distribution, metabolism and excretion,

TABLE. 1
Energy Variation

Time (.ps)	Energy (KJ/Mol)
0	-49106.8
1	-49983
2	-50551.4
3	-50759.4
4	-51172.8
5	-51864.6
6	-51329
7	-51033.7
8	-51339.5
9	-51339.5
10	-50873.6

TABLE. 2

COMPOUND NAME	E.VALUE
Apicidin Inhibitor	-5.1
Trichostatin Inhibitor	**-5.58**
Thiophene Derivative Inhibitor	-5.67
Ketomide,12 Inhibitor	-6.8
PXD 101 Inhibitor	-6.23
LAQ-824 Inhibitor	-5.43
Amino Methyl Pyridine Based, 13b Inhibitor	-5.88
Pyridine Based Compound, 3 Inhibitor	-5.16
Triazole Ligand, 10a Inhibitor	-7.81
FK228 Analogue Inhibitor	-6.14
Benzamide Type Inhibitor, 17	-5.2
4,Phenylimidazole,19 Inhibitor	-5.79
SAHA Inhibitor	-5.3
Thiolate Analogue, 15a Inhibitor	-6.64
NCH-31 Inhibitor	-5.17
Benzamide Type Inhibitor 4	**-7.62**

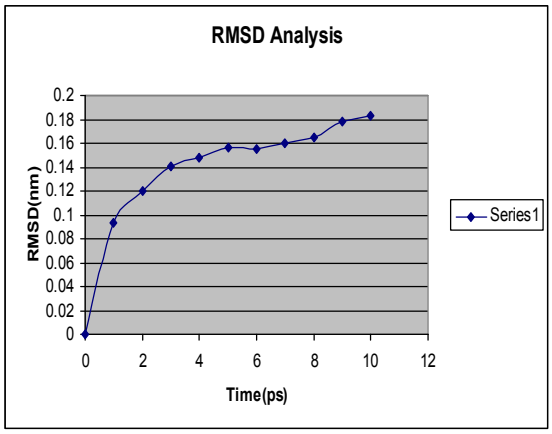

Fig.3. RMSD Analysis Plot for Dystrophin

which are major parts of pharmacokinetics. Numerous in vitro methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, $CaCo_2$ cell model and has been recommended as a reliable in vitro model for the prediction of oral drug absorption. It was also found that Benzamidine inhibitor type 4 satisfied the absorption properties and passed the Lipinski's rule of five (Silvia

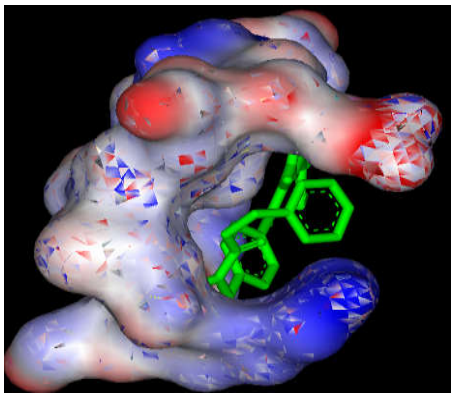

Fig.4. Molecular Surface View of the Interaction between benzamidine inhibitor and dystrophin protein

Miret et al., 2004). The one of the most crucial reasons why a drug discovery fails is the toxicity of the drug candidates. It means that designing drugs with the consideration of their toxicity is very important. Pre-ADMET predicts mutagenicity and carcinogenicity of a compound, helping you to avoid toxic compound. The Ames test (Bruce n. Ames, et al) was TA 100(+S9), TA1535 (+S9), TA1535 (-S9), TA98 (+S9), TA98 (-S9) showed negative results. The carcinogenicity tests which showed positive, where it indicates a positive prediction, inferring that No evidence of carcinogenic activity.

DISCUSSION

The protein dystrophin was modeled using the Modeler 9v1 and was validated using the Procheck were it was found to have 95.1% accuracy in the Ramachandran plot. The superimposed structure and the template RMSD value is calculated using combinatorial extension (CE) algorithm (Shindyalov *et al.,* 1998) which was found to be 0.30A°, the deviation between the template and the modeled protein (Dystrophin) was visualized using Accelrys DS visualizer.

The best 16 active compounds were screened based on the Lipinski's rule of five and docking was performed for the 16 best compounds using Autodock, Out of the 16, the best ligand was found to be Benzamide type inhibitor 4 having energy value -7.62 which is higher than TrichostatinA (-5.67). The best conformation was found to have two bonds with the interaction at ARG114 and MET99 residues. Energy Minimization was performed to remove overlapping atoms. The macromolecular interaction studies and ADME Tox analysis showed that Benzamidine type inhibitor 4 may be possible dug candidate which was found to posses higher inhibition value than the commercial drug TrichostatinA, with less side effect, (Matthew Wetzel *et al.,* 2005).

CONCLUSION

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the market place. Computer – Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug – receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and databases. The Modern drug discovery is characterized by the production of vast quantities of Compounds and the need to examine these huge libraries in short periods of time. The Protein-Ligand interaction

plays a significant role in structural based drug designing. The interactions studies of the modeled protein with the drug compounds showed that Benzamidine type inhibitor 4 having higher affinity value compared to the commercial available drug TrichostatinA, which is a better drug for the treatment of muscular dystrophy.

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