



PFMDR1- A Potential Target to combat the Mutli Drug Resistance in Malaria

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Abstract:

Plasmodium Falciparum Malaria (PFM) infection is a leading source of life killing diseases such as Cerebral Malaria, Nephritis, Hepatic disorder and Severe Anaemia. In the current study, we use computational methods in order to improve our understanding of the complex disease interactions of Plasmodium Falciparum Malaria. Through our work we tried to emphasize the complexity of the disease and the key molecular players involved in its pathogenicity. We found one of the crucial genes named PFMDR1 in this study that proves to play an important role in the diagnosis and treatment of the disease. The protein encoded by this gene has been popularly reported for its drug resistance ability. Therefore, PFMDR1 protein is the preferential choice for inhibition to stop the drug resistance processing. PFMDR1 Gene characterization studies were performed. SNPs responsible for drug resistance and its corresponding Amino Acid change were studied. Mutational analysis and Biological significance of the mutations were analyzed. From the overall study, we found mutation N1042D plays prominent role in drug resistance. Hence, the 3D structure of mutated PFMDR1 protein was modelled. For inhibiting the action caused by the mutation, 120 Natural Chemical compounds which have the anti malarial property were selected. The compounds were screened for Druglikliness and Toxicity. 60 chemical compounds were finally screened. The chemical structures were further subjected to Geometry optimization and Energy minimization for standard confirmation of the chemical structures. MDR1 protein's mutational site was explored to find out the critical interactions for inhibitor binding using molecular docking methodology. After several docking steps, Finally LAPACHOL compound with the binding energy -11.09444Kcal/mol was found to be the potent drug candidate which can efficiently interact with the mutational site thereby inhibiting the phenomenon of drug resistance in Plasmodium Falciparum. It should be noted that these predicted

data should be validated using suitable assays for further consideration.

Introduction:

Infection by the malarial disease is curable and can be preventable but it is still the most important life threatening disease in the world wide. The current Consensus report that there are annually about 500 million clinical cases of malaria, 2-3 million severe attack and 1 million deaths, which is approximately equal 3000 death per day, estimated by the report in African country it takes every 30 second a life. Malaria remains uncontrolled not only in the developed countries but also in developing countries. Few important reasons can be emergence of the drug resistant parasite, widespread use of dichlorodiphenyltrichloroethane (DDT) insecticide resistant mosquito vector and non availability of suitable and effective malaria vaccine. The disease burden is increasing in almost all the tropical countries. Since malaria creates socio-economic problems and also causes large number of deaths, particularly among young children and pregnant women. The situation is becoming more difficult because the most widely used antimalarial drug chloroquine is losing its effectiveness. Plasmodium falciparum from Sub Saharan Africa started showing resistance to this drug in late 1950s which was followed in other parts of the World. In India, the chloroquine resistance was first reported from Assam in 1973[1] and had caused some epidemics since 7-8 years. Sulphadoxine-pyrimethamine is used as a second line of drug to treat uncomplicated chloroquine resistant falciparum malaria cases but recent past it is also noted that this drug is also showing its resistance all over India. Although various other antimalarial drugs are also being used but chloroquine and sulphadoxine - pyrimethamine remains the most widely used drugs. Molecular drug resistance profile of the parasite population consistent with a chloroquine and sulphadoxine-pyrimethamine resistant phenotype strongly indicate that a police

change to artemisinin based combination therapy (ACT) has to be considered in the near future. Standard treatment for malaria is combination therapy artemisinin and sulphadoxine or any other. Artemisinin combination therapy (ACT) and replaced former mono-therapy options in management of uncomplicated malaria as recommended by WHO [2]. This review provides information on the molecular targets for these commonly used drugs, and also on mutations in these target genes which are associated with the development of drug resistance, Resistance against these drugs is being reported from all over the world wide, , *pfcr1*[3], *pf mdr-1*[4,5,6,],(*Chloroquine Resistance*) *dhps*[7,8], *dhfr*[9](*Sulfadoxine Pyremethemine*) , *PfATPase6*[10] (*artemisinin*). High-level chloroquine resistance appears to require alterations in other genes such as *P. falciparum* multidrug resistance (*pfmdr1*) Polymorphisms K76T in *pfcr1* and N86Y, S1034C, N1042D, and D1246Y in *pfmdr1* have been identified as being associated with chloroquine resistance, however the efficacy of this control strategy is hampered by the emergence and spread of drug resistant malaria which is the major challenge in the control of the disease at present. Therefore research efforts into the design and development of new antimalarial drugs, which are safe effective and affordable are of prime importance.

Materials and method:

Using the search terms Malarial Disease mortality and morbidity and roll of PFMDR1 an intensive literature search of scientific papers was done in order to identify genes, proteins and small molecules that relate to MDR1 and its Drug Resistance Action. Finally, peer reviewed articles had been enrolled and combined with data publicly available in the NCBI database 'http://www.ncbi.co.in.' order to further identify proteins involved in Falciparum Malaria and to aid reconstruction of pathways. However, only a few of these applications are described here as examples of the importance of molecular visualization and modelling in structure-based drug design. Complete Gene analysis and SNPs were analyzed using Ensemble Genome Browser and SNP Database. The prominent SNP and the corresponding Amino acid change is traced and the deleterious effect was analyzed. Ab Initio Structure Prediction Method is one of the approaches in Structure modelling which helps in designing the mutated 3D structure of PFMDR1. The receptor protein structure standardization was performed using Argus Lab.

Natural Anti malaria drug candidate study were selected for study. 120 selected chemical compounds were screened for Drug likeliness using Lipinski Rule, Toxicity. 60 chemicals have qualified the above analysis .These drug candidates are further standardized by geometry optimization and energy minimization of the structure. Following are the drug candidates for performing the Docking studies : Mefloquine Compound, Arbotristis, Nyctanthes Arbotristis, Heteratisine, Heterophylline, Benzylheteratisine, Beta —Sitosterol, Caesalpini Pluviosa, Corymbosin Compound, Rohituka compound, kummel compound, Spiro, Artesunate compound, Eclipta prostrate ,Paniculata, Callosobruchus chinensis, Caesalpin J Compound, Violacene compound, N,N Methylenebis, Adenosine Diphosphate, quinidine compound, Apachol Compound, Cinchonine, Proguanil Compound, monorden Compound, Sinefungin Compound, Lumefantrine compound, Amodiaquine Compound, Spirogermanium Compound, Halofantrine Compound, Sulfadoxine Compound, Methylthioadenosine Compound, Acedapsone Copmound, Monorden Compound, Chloroquine Compound, Gedunin Compound, Quercetin Compound, Eupatin, Nauclea pobeguini, Elatin Flavonoid, Silicon Phythalocyanine compound, Tetrandrine Compound, Xanthoxyline Compound, Leiocarpin Compound, Phyllanthus Muellerianus, Cuercetin, Dacryodes Edulis, Azadirachtin, Azadirachtin O,O-Bis(Trimethylsilyl), Azadirachtin O Compound, Azadirachtin H , Azadirachtin P, Gedunin Plant, Latifoline Funtumia, Curcumin Dimethyl Ether, Echitamine, Doxycycline Compound, Primaquine Compound, Dapsone Compound, Quinacrine, Trimethoprim-Sulfamethoxazole, Bredinin Compound, Betulinic Acid, Mefloquine, . Brl 6231 Compound, Artemether Compound, Cycloguanil Compound, Trimethoprim, Tetrandrine, Pyrimethamine, Dihydroquinghaosu, 5'-Methylthioadenosine, Pepstatin, Spirogermanium, Artesunate Compound, Chlorproguanil Compound, Magainin 1 Peptide, Xenopus, Dermaseptin, Cecropin B Protein, Insecta, E 64 – Compound, Dihydroquinghaosu, Pyronaridine, 5'-Deoxy-5'-S-Isobutylthioadenosine, 3-Amino-2-Hydroxy-4-Phenylbutanoic Acid, Piperaquine, 3-Dichloro-6-Trifluoromethyl-9-Phenanthryl-3-(N-Butyl)Aminopropanol Compound , Rv 538 Compound, Cryptolepine, Parvaquone Compound

Results and Discussion:

Gene and Protein Characterization studies of PFMDR1:

The PFMDR1 gene and protein analysis was performed. Using Ensemble Genome and SNP analysis was performed. Based on Mutational studies and protein characteristic features the mutation N1042D is found to be highly favourable and prominent target site for Drug designing. From the Domain analysis and active site prediction we found that the mutational site lies in the functional region ABC Transporters region. Reports suggest that ABC transporter region plays a prominent role in drug resistance. Therefore the mutational site in this region can be of prime importance. The mutational site was identified to be present on the active binding site regions through the binding site analysis tools. From the overall studies the mutational site N1042D was chosen as the most favourable binding site for exploring the interaction with drug molecule.

Ab Initio Structure Prediction:

For designing the mutated PFMDR1, the Ab Initio Structure prediction method was employed. Using the I Tasser Server 5 models of the mutated structure was generated. Based on the C Score (Confidentiality Score) Model 1 was selected for our study.

1. Model C- Score : -1.30
2. Model C-Score : -1.30
3. Model C-Score : -1.44
4. Model C-Score : -1.83
5. Model C-Score : -1.96

Estimated accuracy of model: 0.55 ± 0.15 (tm score), $13.0 \pm 4.2 \text{ \AA}$ (RMSD)

Docking Studies:

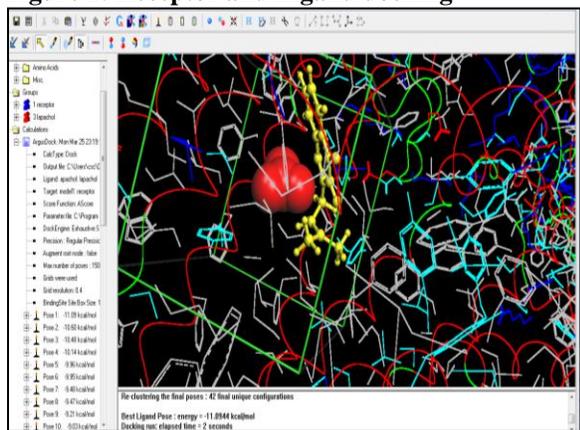
For performing Docking Studies, the mutational site residue Asparagine at position 1042 was selected as the Receptor target site. The 60 screened ligands which are the lead molecules were used for docking. Of the 60 ligands, 45 ligands were found to have no acceptable poses with the target site. 15 ligands were found to dock with the mutational site. The ligand poses were analyzed and a heat map was produced to count H bonds made by the poses to the receptor molecule and count close contacts between the poses and receptor molecule. The best ligand was selected based on the pose binding energy and top score . Docking results indicate that conserved amino acid residue Asparagine 1042 in MDR1 protein play an

important role in maintaining a functional conformation and directly involved in drug binding. The chemical compound Lapachol which has anti malaria property was found to be the best ligand from the table 1 with binding energy -11.09444 . It can be speculated that the conformational changes caused due to mutations at the active site of target protein, may cause alteration in the drug binding ability of the protein which can be rectified by using a suitable ligand.

Table 1: Docking Results

Compound Name	Energy calculation
ARTESUNATE	Best Ligand Pose : energy = -9.47133 kcal/mol Docking run: elapsed time = 3 seconds
LAPACHOL	Best ligand pose: energy = -11.0944 kcal/mol Docking run : elapsed time = 3 seconds
ARTEMETHER	Best Ligand Pose : energy = -8.61369 kcal/mol Docking run: elapsed time = 4 seconds
BREDININE	Best Ligand Pose : energy = -4.81903 kcal/mol Docking run: elapsed time = 5 seconds
CURCUMIN	Best Ligand Pose : energy = -11.1729 kcal/mol Docking run: elapsed time = 23 seconds
CYCLOGUANIL	Best Ligand Pose : energy = -9.80215 kcal/mol Docking run: elapsed time = 3 seconds
DAERYODES	Best Ligand Pose : energy = -10.4659 kcal/mol Docking run: elapsed time = 6 seconds
DAPSONE	Best Ligand Pose : energy = -9.61971 kcal/mol Docking run: elapsed time = 5 seconds
LUMIFANTRINE	Best Ligand Pose : energy = -7.13699 kcal/mol Docking run: elapsed time = 15 seconds
NSC348102	Best Ligand Pose : energy = -6.76534 kcal/mol Docking run: elapsed time = 3 seconds
PROGUANIL	Best Ligand Pose : energy = -7.27408 kcal/mol Docking run: elapsed time = 3 seconds
QUISQUALIC ACID	Best Ligand Pose : energy = -5.3045 kcal/mol Docking run: elapsed time = 9 seconds
XANTHOXYLINE	Best Ligand Pose : energy = -6.91937 kcal/mol Docking run: elapsed time = 4 seconds
ZIDOVUDINE	Best Ligand Pose : energy = -6.73578 kcal/mol Docking run: elapsed time = 3 seconds
CHLOROQUINE	Best Ligand Pose : energy = -10.6403 kcal/mol Docking run: elapsed time = 5 seconds

Figure 1: Receptor and Ligand docking



Conclusion:

With the help of predicted models, the conformational changes due to mutations at the active site residues were determined. On the basis of docking score, it can be interpreted that the strict constraints resulting from such mutations could significantly alter the folding of the mutant protein and or the positioning of the Amino Acid residues. This may readily explain the loss of PFMDR1 activity thereby inhibiting the effect due to conversion of ASN to ASP. Also, how point mutations can dramatically alter the ability of drug binding which eventually may lead to differences among the mutants. Thus, we suggest that the mutant N1042D represented with high docking score may contribute to low-level drug resistance than other mutants. The findings suggest that redesign of the PFMDR1 Protein molecule to improve drug binding may be a viable approach to overcome resistance in especially for mutant with N1042D substitutions. Such studies will be helpful in better understanding about the mechanism of drug resistant. Detection of mutations at the active site of the target protein enables to gain novel insight into the drug-target interactions, leading to the rational design of more efficacious wonder drugs that not only shorten malarial therapy but also can prevent the emergence of drug resistance.

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Reference:

1. Malaria in India.Guidelines for its control. NationalVector BorneDiseaseControl Programe. <http://www.nvbdc.gov.in/malarianew.html>

2. World Health Organization. Development of South-Asia surveillance network for malaria drug resistance. Report on informal consultative meeting. Geneva, Switzerland: World Health Organization, 2008.

3. Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF, 2000.Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. *Nature* 403: 906–909.

4. Babiker HA, Pringle SJ, Abdel-Muhsin A, Mackinnon M, Hunt P, Walliker D, 2001. High-level chloroquine resistance in Sudanese isolates of *Plasmodium falciparum* is associated with mutations in chloroquine resistance transporter gene pfcrt and the multidrug resistance gene pfmdr1. *J Infect Dis* 183: 1535–1538.

5. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LM, bir Singh Sidhu A, Naude B, Deitsch KW, Su X, Wootton JC, Roepe PD, Wellems TE, 2000. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol Cell* 6: 861–871.

6. Foote SJ, Kyle DE, Martin RK, Oduola AM, Forsyth K, Kemp DJ, Cowman AF, 1990. Several alleles of the multidrug resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. *Nature* 345: 255–258.

7. Triglia T, Menting JGT, Wilson C, Cowman AF, 1997. Mutations of dihydropteroate synthase are responsible for sulfone and sulfonamide resistance in *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 94: 13944–13949.

8. Triglia T, Wang P, Sims PFG, Hyde JE, Cowman AF, 1998. Allelic exchange at the endogenous genomic locus in *Plasmodium falciparum* proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. *EMBO J* 17: 3807–3815.

9. Zolg JW, Plitt JR, Chen GX, Palmer S, 1989. Point mutations in the dihydrofolate reductase-thymidylate synthase gene as the molecular basis for pyrimethamine resistance in *Plasmodium falciparum*. *Mol Biochem Parasitol* 36: 253–262.
