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In silico attempt for adduct agent(s) against malaria: Combination of chloroquine with alkaloids of *Adhatoda vasica*

*Shasank S. Swain, Mahesh C. Sahu, Rabindra N. Padhy**

Central Research Laboratory, IMS and Sum Hospital, Siksha 'O' Anusandhan University, K-8 Kalinga Nagar, Bhubaneswar 751003, Odisha, India

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ABSTRACT

With the aim of controlling drug resistant *Plasmodium falciparum*, a computational attempt of designing novel adduct antimalarial drugs through the molecular docking method of combining chloroquine with five alkaloids, individually is presented. These alkaloids were obtained from the medicinal plant, *Adhatoda vasica*. From the obtained individual docking values of important derivatives of quinine and chloroquine, as well as, individual alkaloids and adduct agents of chloroquine with *Adhatoda* alkaloids as ligands, it was discernible that the 'adduct agent-1 with chloroquine and adhatodine' combination had the minimum energy of interaction, as the docking score value of −11.144 kcal/mol against the target protein, triosephosphate isomerase (TIM), the key enzyme of glycolytic pathway. Drug resistance of *P. falciparum* is due to a mutation in the polypeptide of TIM. Moratorium of mutant TIM would disrupt the metabolism during the control of the drug resistant *P. falciparum*. This *in silico* work helped to locate the 'adduct agent-1 with chloroquine and adhatodine', which could be taken up by pharmacology for further development of this compound as a new drug against drug resistant *Plasmodium*.

1. Introduction

Among malaria prevalent Indian states, Odisha was estimated having 22% prevalence of malaria approximately a decade ago of which, about 41% were due to *Plasmodium falciparum*, along with *Plasmodium vivax* as the second most important parasitic species [1]. Prevalent values of *P. falciparum* are reported varying from 40 to 60 to 80 to 100 cases in 1000 people in different states of the Indian *terra firma*, including Assam and adjoining states, among countries of South East Asia [2]. Thus, malaria is endemic in the majority of Indian states, being linked mainly to living conditions of marginalized people, who live below the poverty line in societies of rustics and aborigines as well as, slum ghettoes [3], where there from it spreads all over. Apart from South East Asia, countries at/near the equator, Sub-Saharan Africa and Latin America too have high prevalence of malaria [4], since the tropical climate remains conducive to survival of mosquitoes. For instance in 2012, malaria caused an estimated 627 000 deaths (with an uncertainty range of 473 000–789 000) all over, including African children [4]. Moreover, non-immune travellers from malaria-free areas are remarkably vulnerable to the disease on being bitten by the mosquito *Anopheles*.

* *Corresponding author*. Tel.: +91 9437134982; fax: +91 6742432034.
E-mail address: rnpadhy54@gmail.com (R.N. Padhy).

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The option for malaria treatment is limited to alkaloids (chloroquine and other derivatives of quinine, 8-aminoquinolines, preferably primaquine) from 2 to 3 species of *Cinchona*, as well as, recently introduced artemisinin and derivatives from *Artemisia annua*. In the revised WHO directives since 2001, several regimens of artemisinin-based combination therapy (ACT), with derivatives of artemisinin and/or quinine are in use. In an ACT formulation, one of artemisinin-based compounds, dihydroartemisinin, artesunate and artemether is combined with one of the following drugs, lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine and chlorproguanil/dapsone; but, artemether is the principal compound most often. ACTs are quite promising in treating malaria due to both species of *Plasmodium*, and have an additional public health benefit of reducing the overall malaria transmission. In India particularly nowadays, the revised ACT policy consists the use of 'artemether + lumefantrineis', as drugs in seven North Eastern states. In other states, the ACT formulation 'artesunate + sulphadoxinepyremethamine' is used for *P. falciparum* cases. And an ACT with chloroquine is totally effective for *P. vivax* cases. In India, the current drug policy of ACT in case of drug resistant malaria consists of sulfadoxine and pyrimethamine at 25 and 1.25 mg/kg body weight, respectively on the first day, along with artesunate (a semi-synthetic derivative of artemisinin) at 4 mg/kg body weight daily for 3 days [5].

However in 2008, soon after the start of wide use of artemisinin in 2007, artemisinin resistance in *P. falciparum* was first reported from the Cambodia–Thailand border and, it is now prevalent in Cambodia, Myanmar, Thailand and Vietnam. In India too, like other South East Asian countries, artemisinin resistant *P. falciparum* cases are detected in 10% malaria cases. Eventually, the consternation of the future failure of the current ACT stems from the rapid spread of artemisinin resistance in *P. falciparum*. Moreover, in the pre-referral treatment for severe malaria, the role of quinine and chloroquine has now become questionable due to the resistance in *P. falciparum* and *P. vivax*; nonetheless in India, quinine and derivatives continue to play a critical role in the control. In the perspective of comparative effectiveness research, attempts for locating suitable compounds to overcome the drug resistance in malaria could be undertaken, with the modern tools of bioinformatics with the spectacular advances in molecular biology of protein structure and phytochemistry, to state contemplatively. Currently developed *in silico* attempts presented here, using two phytochemicals as a ligand in a molecular combination against a parasitic protein as the target, might open a novel/alternate option with the combined phytochemicals as adduct drug(s) for the control of malaria.

Specifically in those regions where *Cinchona* alkaloids are used, chloroquine resistance in *P. falciparum* increased to a greater proportion [6], for example at 49% of the total burden in 2007 in India was recorded [7]. *Cinchona* alkaloids as drugs cause moratorium of the synthesis of a key enzyme, triosephosphate isomerase (TIM) in the glycolytic pathway of the parasite in its intra-erythrocytic stage; TIM catalyzes the reversible isomerization of dihydroxy-acetone 3-phosphate to D-glyceraldehyde-3-phosphate. The evolutionary mutation of the TIM in *P. falciparum* is one of the major causes of drug resistance [6,8,9]. The amino acid glutamate is replaced by glutamine in the mutated form, at the position-97 of the polypeptide chain in the normal TIM [10]. The structure and biochemistry of TIM are well documented [8,9,11] and TIM can be used as the suitable target protein for drug-designing against *P. falciparum*. Since, resistance of *P. falciparum* is due to a mutation in the polypeptide of TIM, the moratorium of mutant TIM would disrupt the metabolism during the control of the drug resistant *P. falciparum*.

Side effects of the presently used antimalarial drugs are summarized (Table 1). Ghoulish strains of *P. falciparum*, causing frequent cerebral malaria are resistant to drugs, chloroquine and quinine derivatives in several endemic areas [6,7,18], resulting in treatment failures. Furthermore, the cavalcade of critical side-effects, such as the induction of hypoglycemia and neurological disorders of the existing antimalarial drugs, demands search for novel, effective and well-tolerated drugs.

A comparative effectivity-account of drugs, chloroquine, quinine and quinine-derivatives on the normal and mutated TIM species for the inhibition of cellular energy production is described in this paper, using the molecular docking tool of bioinformatics. The formation of adduct agents of chloroquine and individual alkaloids from the Indian antimalarial plant, *Adhatoda vasica* Nees (family, Acanthaceae) was examined, against normal and mutant TIM, in protein–ligand interactions, wherein adduct agents of chloroquine and individual alkaloids serve as ligands against TIM, the larger target protein. *A. vasica* also has unique alkaloids, adhatodine, anisotin, vasicine, vasicinone and vasicolinone for which probably, this plant has been in use for the control of malaria [19–21]. Consequently, involving the enzyme TIM in the further drug development cascade would be the prudent approach. The present work on *in silico* computation would help locating a suitable adduct agent without the hit-and-miss method, generally followed in drug targeting attempts *in vivo* by apothecary. Secondly, adducts (conjugates) used are regarded as adjuvants, *i.e.*, substances that modulate the effect of the active substance, however by themselves are not active. In this study, *Adhatoda*-alkaloids lent themselves as adduct molecules in modulating the effect of quinine and its derivatives individually as coveted adduct-combinations for mutated TIM of drug resistant *P. falciparum*, computationally.

2. Materials and methods

Crystallographic protein structures of both normal TIM and the mutant variant of *P. falciparum* were selected and retrieved from protein databank (PDB) (http://www.rcsb.org); the PDB ID: 1YDV is of the normal TIM and the PDB ID: 3PSW is of the mutant TIM, which were used for docking attempts (Fig. 1). Three-dimensional structures of the current drugs, quinine and chloroquine along with quinine derivatives, acetylquinine, quinine ethylcarbonate, quinine phosphate and quinine valerate as well as, the alkaloids of *A. vasica*, adhatodine, anisotin, vasicine, vasicinone and vasicolinone were retrieved from databases, PubChem (http://www.ncbi.nlm.nih.gov/pccompound) and ChemSpider (http://www.chemspider.com/). All downloaded compounds were converted from .sdf (dot sdf) to .pdb (dot pdb) files, to