



Repurposing Antimalarial Artemisinin against Candidiasis

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Accepted on: 15-03-2015; Finalized on: 30-04-2015.

ABSTRACT

Currently available antifungals are inadequate with reports of new multidrug-resistant strains. Hence promising novel antifungals and combination therapies are urgently needed. Drugs derived from medicinal plants have shown diverse pharmacological activities with pleiotropic mode of actions. Traditional Chinese medicine Artemisinin (*qinghaosu*, sweet wormwood), a potent antimalarial drug, has shown antihepatotoxic, antibacterial, antifungal, antitumor and antioxidant activities. Repurposed drugs, new uses for existing or abandoned drugs, approach helps to develop second-generation of drugs. Today repurposing drug strategy has gained widespread recognition as a drug development approach due to the increasing demand and short supply of medicines globally. Thus it is a promising proposition to recognize cheaper and synthetic, drugs as novel antifungals and their subsequent use in combination therapies. The structurally dissimilar drugs that targets different biochemical pathways in the pathogen enhance the efficacy and reduce chances of development of drug-resistance against them. In the present review, pharmacodynamics of artemisinin is highlighted as an antifungal agent for its future use.

Keywords: Artemisinin, Antimalarials, Repurposing drugs, Candidiasis, Antifungals.

INTRODUCTION

Globally herbal medicines have been used for traditional treatment of various diseases and ailments. Artemisinin (ART) and its derivatives (Figure 1) for now are the most potent and effective antimalarial drugs available, and clinical resistance has not yet been reported. Artemisinins in antimalarial chemotherapy provide a fascinating opening for development of new antimalarial combinations. The emergence of the drug-resistant strains is influenced by many mechanisms and depends upon numerous intertwined factors such as inherent susceptibility of the pathogen to drug pressure, inadequate dose routines, infection intensity (acute or chronic), unnecessary drug administration and abnormal pharmacokinetics.¹ In response to the past experience of global threat of multidrug-resistant *Candida* strains, there are convincing rationales to consider that resistance to the available antifungal drugs can be reduced or protected by cautious use of synergistic antifungal combinations or by repurposing drugs. The current trends certainly suggest that sooner or later there will be an emergence of resistant-strains against the currently used anti-*Candida* drugs or its combinations. Thus at the present moment it is essential to search for new, inexpensive, synthetic and potent repurposed drugs (compounds) that can be used as partner drugs (compounds) with standard recommended antifungals. These synergistic or additive combinations would help to reduce the dependence on current antifungals without compromising on their potency to cure fungal infections. The concept of drug combination involves the use of two (or more) synergistic or additive drugs that are structurally different and have

independent mode of inhibitory action on various biochemical targets (or pathways) within the pathogen. Thus such combinations firstly augment the efficacy of individual agents and secondly provide a mutual protective shield to each other from development of resistant strains against them.

The genus *Artemisia* of the family Astraceae (Compositae) is one of the largest and most widely distributed genera consisting of about 500 species. Few key phytochemicals found in this genus are terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes. Various plant species of the genus *Artemisia* have shown a vast array of potent medicinal properties such as antimalarial, antihepatotoxic, antibacterial, antifungal, anticancerous and antioxidant activities.²

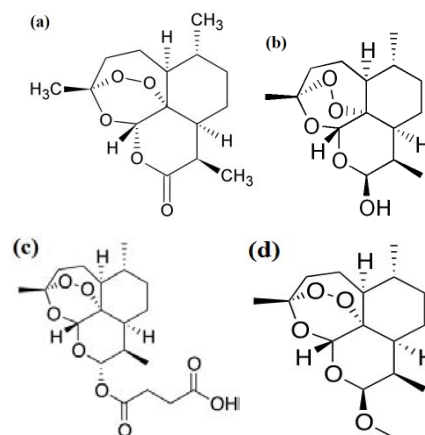


Figure 1: Chemical structure of (a) Artemisinin (Qinghaosu or ART), (b) Dihydroartemisinin (Artemimol or DHA), (c) Artesunate and (d) Artemether. (Source: Wikimedia Commons)

ARTEMISININ: A POTENT ANTIMALARIAL DRUG

The history of using artemisinin as herbal extract for treatment of malaria like fever, dates back to more than 2000 years as a traditional chinese medicine. ART and its derivatives are the most commonly recommended antimalarial drug (as monotherapy or artemisinin combination therapy) by World Health Organization.³⁻⁴ Clinical resistance to ART or its derivatives has not yet been reported while most of the other antimalarials (such as quinine, chloroquine, sulphadoxine, pyrimethamine) has fallen to resistance globally.⁴

ART, known as qinghaosu, is a sesquiterpene lactone compound extracted from the *Artemisia annua* (sweet wormwood) leaves (Figure 2) and has been used as active antipyretic herbal remedy in China for nearly 2000 years.^{3,5,6} This peroxide-bridged sesquiterpene lactone has no nitrogen (N) atom, a distinct feature from quinine-another age-old antimalarial agent (Figure 1). The characteristic peroxide lactone structure is crucial for artemisinin's anti-plasmodial activity.⁶



Figure 2: *Artemisia annua*, a herb traditionally used for fever treatment, contains antimalarial ingredient (Artemisinin) in its fern-like leaves. (Source: Wikimedia Commons)

While the desoxyartemisinin, artemisinin that lacks the endoperoxide bridge, is almost ineffective in parasite inhibition.⁷ ART exert its antimalarial property by disrupting the calcium homeostasis within the intraerythrocytic parasite thus promoting release of Ca^{2+} ions from intracellular stores (endoplasmic reticulum) and inhibiting the metabolic enzyme –sarco/endoplasmic reticulum Ca^{2+} ATPase (also referred as SERCA or PfATP6)-leading to death of malarial parasite.⁷ Few of the potent ART derivatives clinically recommended³ are artemether, artesunate and arteminol (β -dihydroartemisinin, DHA) (Shown in Figure 1).

Artemisinin Combination Therapies (ACT), rather than ART alone (monotherapy), has been strongly advocated by WHO for effective malaria treatment across the globe to combat the menace of drug-resistance.⁴

REPURPOSING DRUGS: A NOVEL THERAPEUTIC APPROACH

Absence of novel antifungal drugs and multi-drug resistant fungal-strains against the conventional drugs is making the immune-compromised individuals vulnerable to various opportunistic infections such as candidiasis. With the advent of advance molecular biology and pharmacology studies, many approaches have become available to search for new therapies essentially specific for the infection, low-priced, simple to use, multi-targeted with no drug-resistant strains reported against them. The urgent need for new avenues of therapeutic management and new era of prophylactic treatment has shown few plausible approaches such as development of analogs of existing drugs, natural products, compounds active against other diseases (also referred as 'repurposing drugs' strategy) and novel synthetic compounds active against new pathogen targets.^{8,9}

A 'repurposing drugs' approach is to identify new pharmacological uses for existing drugs, that are developed as therapeutic agents for other diseases, and its subsequent testing for successful discovery of new therapies.^{8,9} Pharmacologist and Nobel laureate James Black famously said that one of the most productive approach for drug discovery is to start with an old drug.¹⁰ The major advantage of drug repositioning is its known pharmacokinetics, human safety profiles and US Food and Drug Administration (FDA) approval for human use, thus bypassing initial phase trials (Phase 0 'micro-dosing' trails and Phase 1 'pharmacologic' trails). The evaluation of 'repurposed' drugs from phase II clinical trials firstly saves almost 40% of the overall drug-development cost and secondly eliminates the preclinical animal studies, toxicological and pharmacokinetic phase studies.¹¹ Reduction in developmental cost and drug-launch time with previously compiled pharmacokinetic, toxicology and safety statistics has highlighted the 'repurposed-drug' approach as a rational and cost-effective strategy among global pharmaceutical industries. In the recent years, the repurposing approach has successfully revealed new uses for old drugs and few of the examples are briefly discussed below.^{8,9,12-14}

- Miltefosine, a phospholipid drug, developed as an anti-cancerous agent was subsequently found effective and safe in treatment of visceral leishmaniasis. The precise antileishmanial action of miltefosine remains unclear.
- Chlorproguanil-Dapsone, a combination of analog of proguanil with dapsone, is an old dihydropteroate synthase (DHPS) inhibitor. Dapsone, a sulfone drug, is of importance in the treatment of leprosy.¹⁵
- Heat-treated Amphotericin B (HT-AMB), a super-aggregated form of AMB, and Clotrimazole (CLT) has shown potent antiplasmodial activity *in vitro*.^{16,17} Both AMB and CLT are FDA-approved synthetic compounds used as antimicrobial agents for the

treatment of serious fungal infections. Further *in vitro* fixed-dose combinations of ART with CLT or HT-AMB against *Plasmodium falciparum* has been reported to be synergistic to additive interactions.¹⁸

- Thalidomide drug was used for the treatment of erythema nodosum leprosum, a rare form of leprosy. Later thalidomide was repurposed for multiple myeloma.
- The beta-blockers propranolol (Inderal) and metoprolol (Toprol-XL), commonly used for treating hypertension, was successfully repurposed for the treatment of congestive heart failure (CHF).
- Leucovorin, used for high-dose methotrexate rescue, showed synergistic interaction with fluorouracil (5-FU) in treating colorectal cancer. Levo-isomer, levo-leucovorin, a second-generation agent is now prescribed for both methotrexate rescue and colorectal cancer treatment.
- Mifepristone used for blockage of uterine progesterone receptors, that is chemical termination of early pregnancy, was repurposed to manage hyperglycemia in persons with Cushing's syndrome.
- Stylianou *et al.*, 2014 screened 844 drugs of which 26 agents showed anti-*Candida albicans* activity.¹⁹ Of these twenty-six drugs, 12 agents were standard antifungal drugs, 7 were off-target drugs (non-antifungal drugs) which possesses anti-*Candida* activity and the remaining 7 agents were newly-identified off-target drugs (amonaftide, tosedostat, megestrol acetate, melengestrol acetate, stanozolol, triflurperidol and haloperidol).

WHY ARTEMISININ AGAINST CANDIDIASIS?

Serious life-threatening infections are increasing globally that are generally caused by well-known opportunist pathogens such as *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.²⁰ The increase in morbidity and mortality associated with serious fungal infections are largely found in immunocompromised patients who have undergone organ transplantation, blood or marrow transplantation, HIV infected- AIDS patients, or patients having undergone immunosuppressive therapy and cancer chemotherapy.^{21,22} Diverse and increasing range of opportunistic mycoses along with complexity of fungal infections is threatening current diagnostic and therapeutic approaches. About 17 different *Candida* species have been reported, of which *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* acts as pathogens in the bloodstream fungal infections, a major cause of mortality in hospitalized patients.^{23,24} Most of the recommended antifungal drugs have major constraints as they mainly inhibit cell wall or ergosterol biosynthesis pathways in the pathogen, thus the possibility of drug-resistance against the monotherapy

drug or its combination is greater. These opportunistic pathogens have developed intrinsic or acquired resistance to commonly recommended azoles and other antifungal drugs.^{20,24} Thus any new, safer, and potent antifungal drugs acting on the opportunistic pathogen such as *Candida albicans* is of great importance. Reports suggest that *Artemisinin annua* extract apart from antimalarial property²⁵ also possesses potent anti-inflammatory, antipyretic²⁶, anticancer²⁷, antifungal²⁸, antiprotozoal²⁹, and antitrypanosomal³⁰ activities. *In vitro* antifungal activity of artemisinin derivatives against *Candida albicans* has been previously reported.^{31,32} Novel synthesized artemisinin derivatives or endoperoxides has also shown good fungistatic (block growth by budding) to fungicidal (inhibiting the morphogenetic transformation of *C. albicans*) activities.^{32,33} In a recent proteomic or microarray study, researcher have shown that artemisinin targeted the oxidative phosphorylation pathway (64 kDa mitochondrial NADH dehydrogenase) in *Aspergillus fumigatus*, a pathogenic filamentous fungus, which is a unique intracellular target previously not inhibited by any commonly used antifungal drugs.³⁴ The result were consistent with the previous reports which suggested that deletion of either NDE1 or NDI1 genes (that encodes for mitochondrial NADH dehydrogenases) resulted in artemisinin-resistant yeast cells.³⁵ Artemisinin treatment to the fungal-pathogen *A. fumigatus* effected its cell wall and associated proteins/enzymes (conidial hydrophobin B protein, cell wall phiA protein, 1,3-beta-glucanosyltransferase- Gel2), ergosterol biosynthesis pathway (ERG6 and coproporphyrinogen III oxidase, HEM13), transport proteins and cell stress proteins.³⁴ Further artemisinin-treated *Saccharomyces cerevisiae*, budding yeast cells, showed depolarization of mitochondrial membrane and generation of reactive oxygen species.³⁵ In another report it was shown that artemisinin targeted the *S. cerevisiae* calcium channels (Ca²⁺ - ATPases) - Pmr1p and Pmc1p and targets two biochemical machineries within the pathogen – firstly through specific binding to calcium channels and secondly via generation of free radicals in the mitochondria.³⁶ Although the exact mode of action of artemisinin or its derivatives against *C. albicans* remains elusive but it would be an attractive proposition to use artemisinin as an antifungal drug partner on these opportunistic fungal pathogens.

CONCLUSION

Repurposing drug approach is an attractive alternative to search for potent antifungal agents. Artemisinin, a potent antimalarial, has showed wide spectrum antimicrobial activity and targets essential biochemical processes within the opportunistic fungal pathogens such as oxidative phosphorylation pathway. Synergistic or additive combinations of artemisinins with standard antifungal agents provide a rationale approach for the identification of new antifungal therapies in the near future.



Acknowledgments: AB, NK and HK are thankful to Ramjas College, University of Delhi and NB is thankful to Dyal Singh College, University of Delhi for the guidance and motivation.

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Source of Support: Nil, **Conflict of Interest:** None.

