Commentary

Combining traditional medicine and modern chemistry to fight malaria

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Provenance: This is a Guest Commentary commissioned by Section Editor Hongcheng Zhu, MD, PhD (Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Comment on: Herman JD, Pepper LR, Cortese JF, *et al*. The cytoplasmic prolyl-tRNA synthetase of the malaria parasite is a dual-stage target of febrifugine and its analogs. Sci Transl Med 2015;7:288ra77.

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A combination of traditional medicine and modern chemistry has provided a promising new lead in the search for new antimalarials. Using a rational chemical approach, Herman and colleagues (1) have identified the target of febrifugine, the active component in the ancient traditional medicine changshan, and modified the compound such that its side-effects are greatly reduced while the anti-malarial activity is maintained.

Malaria remains an important health concern worldwide, despite significant advances in the fight against this disease. Malaria is caused by parasites of the genus *Plasmodium*, of which *Plasmodium falciparum* causes the most severe disease and nearly all of the fatalities in humans. Parasites are introduced into a host by a mosquito bite, after which they infect the liver. Following replication in the liver, parasites capable of infecting erythrocytes are released—it is this stage of the life cycle that is responsible for the disease symptoms. A subset of the erythrocytic parasites will convert to gametocytes, which can spread the disease after being taken up by a mosquito during a blood meal. Over the past 15 years, the reported toll of malaria has decreased 60% to ~438,000 lives lost per annum, according to the most recent WHO report (2). This decrease is in no small part the result of the widespread use of the antimalarial drug artemisinin (and derivatives thereof), used in combination with a second antimalarial. However, these gains are now under threat by the emergence of parasites resistant against artemisinin (3). Hence, development of new antimalarial drugs is of great importance.

Traditional medicine has been a source of many drugs, including antimalarials. Famously, the antimalarial compounds quinine and artemisinin are derived from bark of the *Cinchona* tree and the *Artemisia annua* tree, respectively. Tu Youyou, the discoverer of artemisinin, was awarded the Nobel Prize in Physiology or Medicine in 2015 for her important work on artemisinin. Less well known is changshan, an extract of the herb *Dichroa febrifuga*, which was used already 2,000 years ago to treat the fevers induced by malaria (4). The active ingredient in changshan, febrifigune, was extracted in the 1940's (5,6). Although effective against malaria, the severe side effects (including nausea and vomiting) of changshan and purified febrifugine have prevented their development as an antimalarial (4,7). Several attempts have been made in the past to decrease the side effects through chemical modification, but none of them have been successful (7). Now Herman and colleagues describe two major advances in the development of derivatives of febrifugine as anti-malarial compounds: the identification of its target in the parasite and a modification of the compound that greatly decreases the side effects.

To discover the target of febrifugine, Herman and colleagues cultured *P. falciparum* parasites in the presence of halofuginone, a derivative of febrifugine, until resistant parasites arose. Whole genome sequencing of two independent clones revealed two different mutations in the same codon of the gene that encodes the cytoplasmic

prolyl-tRNA synthetase. This enzyme charges tRNA(Pro) with proline and hence is essential for protein synthesis. To verify that this enzyme is the direct target of halofuginone the authors took advantage of the finding that the yeast *Saccharomyces cerevisiae* is not sensitive to halofuginone. They constructed *S. cerevisiae* strains in which the only functional prolyl-tRNA synthetase was either the *S. cerevisiae* enzyme, the wildtype *P. falciparum* enzyme or the halofuginone-resistant *P. falciparum* enzyme. Consistent with the enzyme being the target of halofuginone, only parasites carrying the *S. cerevisiae* or the halofuginone-resistant *P. falciparum* enzyme, but not the wildtype *P. falciparum* enzyme, grew in the presence of the drug. The authors further showed that halofuginone treatment of *P. falciparum* induces an amino acid starvation response in the parasite, further linking halofuginone with the process of protein synthesis.

Important for the development of any drug is an understanding of the binding of the compound to its target at a molecular level. Modeling of the interaction of halofuginone with the prolyl-tRNA synthetases from *S. cerevisiae* and *P. falciparum*, structures of which have recently been solved (8,9), revealed a potential molecular mechanism of the binding of the drug to its target, and insight into why the drug only inhibits the *P. falciparum* enzyme but not the yeast enzyme.

Many derivatives of febrifugine have been synthesized over the years, including more active compounds, such as halofuginone (10). However, active derivatives still produced the same side effects as febrifugine (7). Analyzing the chemical structures of halofuginone and febrifugine, the authors reasoned that a central ketone functional group could act as a reactive group, leading to side effects that are independent of its activity against the prolyl-tRNA synthetase enzyme. As previous work had shown that derivations lacking this ketone are inactive (11), the authors replaced the ketone with a secondary alcohol, thereby maintaining the ability of this part of the compound to interact with the prolyl-tRNA synthetase. This new molecule, named halofuginol, had a very similar *in vitro* activity against blood stage *P. falciparum* compared to febrifugine, with EC_{50} values of 5.8 and 4.0 nM, respectively. In addition, halofuginol also killed liver stage parasites of the model rodent malaria parasite *Plasmodium berghei in vitro*. When the authors next used the *P. berghei* model to investigate the efficacy of the compound *in vivo*, they found that the parasitemia in *P. berghei*-infected mice treated with halofuginol, either orally or intraperitoneally, was >99% lower than in control animals. Importantly, at the dose used, 25 mg/kg, none of the side effects of febrifugine were detected, indicating that the chemical modification had successfully removed the off-target effects while maintaining the anti-malarial activity. With this, the authors showed that halofuginol is active against asexual blood stage and liver stage parasites similar to fubrifigone, without the side effects.

The combination of traditional medicine and modern chemistry has thus provided a very promising new starting point for the development of a novel anti-malarial. As the authors point out, much remains to be done, such as further investigation of the pharmacokinetics and the development of derivatives with a lower affinity for the human orthologue of prolyl-tRNA synthetase. Also, efficacy against gametocytes needs to be tested. Efficacy against this stage of the parasite lifecycle is considered an important aspect of any new anti-malarial drug, as it would prevent host-to-host transmission of the disease. However, despite these outstanding questions, the anti-malarial drug pipeline will always welcome a new compound.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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