Role of US Military Research Programs in the Development of US Food and Drug Administration–Approved Antimalarial Drugs

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US military physicians and researchers helped identify the optimum treatment dose of the naturally occurring compound quinine and collaborated with the pharmaceutical industry in the development and eventual US Food and Drug Administration approval of the synthetic antimalarial drugs chloroquine, primaquine, chloroquine-primaquine, sulfadoxine-pyrimethamine, mefloquine, doxycycline, halofantrine, and atovaquone-proguanil. Because malaria parasites develop drug resistance, the US military must continue to support the creation and testing of new drugs to prevent and treat malaria until an effective malaria vaccine is developed. New antimalarial drugs also benefit civilians residing in and traveling to malarious areas.

Development of drugs to prevent and treat malaria is a US military priority for several reasons. The infection can render military personnel unable to fight and can cause life-threatening disease. Insect repellent and bednets do not guarantee protection. A protective vaccine does not exist. Troops may develop malaria after leaving malarious areas because of inadequate prophylaxis or poor prophylaxis compliance. Mosquitoes capable of transmitting malaria exist in the United States, and returning troops can transmit it to others.

Malaria had a major impact in the Spanish-American War, the Pacific and India-Burma-China theaters in World War II, and the Vietnam War. Malaria also afflicted troops in the Korean War and Operation Restore Hope in Somalia [1, 2]. In Liberia in 2003, of 290 troops who stepped ashore during peacekeeping operations, 80 (28%) developed malaria, and 69 (44%) of the 157 who spent at least 1 night ashore were afflicted. Five malaria-infected marines developed severe disease requiring intensive care unit support [3].

Quinine profoundly influenced history, because it enabled missionaries, explorers, colonists, and militaries to travel and live where malaria was endemic (including the United States...
The drug was commonly used by the US military by 1830. During the Second Seminole War in Florida (1838–1842), Dr. Benjamin Harney, a career army medical officer, demonstrated the efficacy of large doses of quinine to treat remittent fevers [6]. This led to improved results with quinine by military physicians around the globe. During the US Civil War, the Union Army used >25,000 kg of quinine or other cinchona products. Quinine was key to the completion of the Panama canal, because it prevented malarial illness in canal workers [7].

Efforts to synthesize quinine were started in 1856 by the British chemist William Henry Perkins, but this goal was not accomplished until 1944 and has never been achieved on a commercially economic scale. Charles Ledger and Manuel Manani found a variety of cinchona (Cinchona ledgeriana) with a high quinine content and sold seeds of this tree to the Dutch government in 1865 after the British rejected the offer. Dutch plantations in Java were soon producing 97% of the world’s supply of quinine, which was ~10 million kilograms a year by the 1930s [5]. Quinine was eventually approved in the United States as an oral drug (after the US Food and Drug Administration [FDA] was created), but an intravenous form of quinine was never approved.

Quinidine is a natural component of cinchona bark that was first described in 1848 by Van Heymingen and was prepared and given its present name by Louis Pasteur in 1853. In approximately 1918, W. Frey noted that patients with auricular fibrillation taking quinidine for malaria developed regular heart rhythms. The drug was originally approved for use in the United States as a drug to treat cardiac dysrhythmias and was manufactured by Lilly. The intravenous formulation was noted to be a life-saving remedy for severe malaria (ironically, quinidine must be given in intensive care unit settings because of cardiotoxicity) [8]. In 1991, the FDA granted labeling revision of quinidine for antimalarial purposes [9]. Intravenous quinidine is the only approved drug for treatment of severe malaria available to US troops and civilians, but continued availability is uncertain because newer cardiac drugs are more frequently used [10]. The US Army is developing an intravenous formulation of artesunate—another naturally derived drug with long-recognized antimalarial properties—to treat severe malaria.

**CHLOROQUINE**

Quinine is an effective treatment, especially when given with other drugs for certain types of malaria, but adverse effects ("cinchonism") and a short duration of action make it a suboptimal prophylactic agent. Synthetic antimalarial work was pioneered by the German scientist Paul Ehrlich. In 1891, while studying aniline dyes to develop tissue staining methods, he observed that methylene blue not only stained malaria parasites but killed them. He cured 2 cases of malaria using methylene blue—the first time a synthetic drug was used to treat humans.

During World War I, countries producing quinine were controlled by anti-German forces. Projected quinine shortages and the need for long-acting prophylactic drugs prompted an effort by German companies to synthesize antimalarial compounds. Bayer, a German dye company, soon became a leading pharmaceutical company, at which chemists and biologists assembled to develop synthetic antimalarials using methylene blue as a prototype. In 1932, German scientists Mietzsch, Mauss, and Kikuth reported synthesis of an antimalarial based on another dye, 9-amino acridine, later known as atabrine. In 1938, the US Army received samples of atabrine from Winthrop Stearns, a sister company of the German conglomerate IG Farben. US service members in Panama participated in clinical trials to test the efficacy of atabrine, but Germany controlled the manufacturing of this drug until scientists in the United States successfully manufactured it in 1941. Concerns about skin yellowing and Japanese propaganda suggesting that atabrine caused impotence created noncompliance after it became available for service members. Dosing studies were undertaken with US military personnel at Fort Knox, and Neil Fairly, an Australian military physician, showed that a daily atabrine regime was effective in preventing malaria in volunteer Australian troops. Partial control of malaria (achieved via rigidly imposed use of atabrine beginning in 1942 and use of the insecticide dichlorodiphenyltrichloroethane [DDT] beginning in 1943) was an important factor in Allied success in World War II.

The Allied push to synthesize antimalarial agents during World War II was prompted by Japan’s seizure of Java in 1942. At the time, 90% of the world’s quinine came from Java. Allied interest in chloroquine followed the capture of German supplies of an analogue called sontoquine in Tunis in 1943. Chloroquine and sontoquine had been patented in the United States in 1941 by the Winthrop Company, which had a cartel agreement with IG Farbenindustrie, their original manufacturer, but drug development had stalled [5]. Chloroquine (which can safely be used by pregnant women and children) rapidly controlled clinical symptoms of susceptible malaria with minimal toxicity and was useful as a once-weekly prophylactic drug. Chloroquine, a 4-aminoquinoline, was approved by the FDA in October 1949 [11]. Multiple pharmaceutical companies were involved in its development, including Winthrop, Abbott, E. R. Squibb and Sons, Sharp and Dohme, and Eli Lilly and Company. However, the emergence of chloroquine-resistant *Plasmodium falciparum* and *Plasmodium vivax* have rendered this drug less useful.

**PRIMAQUINE**

Primaquine, derived from methylene blue, is a structural analogue of pamaquine, which was produced in Germany in 1926. Pamaquine is too toxic for clinical use. Primaquine (synthesized by
Robert Elderfield of Columbia University) is in the 8-aminooquinoline class of antimalarial agents. This class demonstrates potent activity against hypnozoite and other liver forms of malaria.

The US Army began development of primaquine in 1944 and undertook large-scale safety and efficacy studies in the early 1950s, when relapsing *P. vivax* malaria emerged as a major problem in veterans returning from the Korean War. Compliance with chloroquine prophylaxis was good, so malaria symptoms developed only after troops departed from Korea. Dr. Alf Alving (University of Chicago) led a research team under contract to the US Army that evaluated the safety and efficacy of new antimalarial agents in inmate volunteers at the Illinois State Penitentiary. Primaquine prevents relapse from *P. vivax* and *Plasmodium ovale* hypnozoites and therefore cures relapsing malaria [12]. Primaquine’s short half-life (4–6 h) requires daily administration for 14 days. During the Korean War, primaquine was given as directly observed therapy during the voyage from Korea to the United States. At the time of FDA approval (January 1952 for military use and August 1952 for civilians), it was recognized that 15 mg/day would not cure malaria due to all strains of *P. vivax* (e.g., the Chessen strain), but higher doses were not recommended because of association with hemolytic anemia in persons of African descent.

Subsequently, hemolytic anemia associated with primaquine was attributed to glucose-6-phosphate dehydrogenase (G6PD) deficiency (most common in persons of African, Mediterranean, Middle Eastern, and Southeast Asian descent) [13]. Reliable tests for measuring G6PD level are now available, and the present primaquine dosage recommendation, according to the US Centers for Disease Control and Prevention (CDC), for non–G6PD-deficient patients is 30 mg/day for 14 days for presumptive treatment of relapsing malaria (terminal prophylaxis) [14].

Staff at the Walter Reed Army Institute of Research and the Naval Medical Research Center reviewed extensive data and concluded that primaquine could be useful for malaria prophylaxis. The Navy laboratory in Jakarta subsequently completed a pivotal Phase 3 efficacy study. The CDC now recommends primaquine as a possible option for prevention of malaria [15, 16].

Tafenoquine (WR 238605), an 8-aminoquinoline analogue of primaquine with a half-life of 2–3 weeks, is under development by the US Army in collaboration with GlaxoSmithKline for prevention and treatment of *P. falciparum* and *P. vivax* malaria [17].

**CHLOROQUINE-PRIMAQUINE**

Chloroquine-primaquine was used successfully by United Nations troops during the Korean conflict. Chloroquine and primaquine were not available in a single fixed-dose combination tablet until December 1969, when regulatory approval of combination tablets manufactured by Sanofi-Synthelabo was the first major accomplishment of the Division of Experimental Therapeutics at Walter Reed Army Institute of Research, established in 1961 to develop new antimalarial drugs. Chloroquine-resistant malaria was noted in Colombia in 1959 and later in Thailand and Vietnam. US troops in Vietnam experienced ~81,000 cases of malaria, with 1.4 million malarial sick-days and 133 deaths. Discontinuation of DDT use because of insecticide-resistant mosquitoes and adverse environmental effects contributed to the problem.

Chloroquine-primaquine (commercial name: Aralen phosphate with primaquine phosphate; Winthrop-Stearns) was given to troops during the Vietnam War, but it was ineffective against chloroquine-resistant malaria. The combination drug is no longer commercially available, although each drug component is still available separately.

**SULFADOXINE-PYRIMETHAMINE**

The antimalarial activity of sulfa drugs, which block nucleic acid synthesis required for malaria parasite multiplication, was recognized in the 1940s [18]. Because sulfadoxine and pyrimethamine interfere with folic acid synthesis differently, the combination results in a higher degree of anti–folic acid activity, compared with monotherapy. Pyrimethamine was first created by George Hitchings and others at Burroughs Wellcome in the United States in 1950 as part of an effort to develop anticancer agents, and it was approved as an antimalarial drug in Britain in 1951. Pyrimethamine was widely used with chloroquine in the World Health Organization (WHO) control programs of the 1960s, and pyrimethamine-dapsone (Maloprim; Wellcome) was used for treatment and prophylaxis of chloroquine-resistant malaria in Vietnam. However, use of dapsone was associated with infrequent but severe agranulocytosis and methemoglobinemia [19]. Therefore, US Army scientists explored other folic acid–blocking drug combinations. Walter Reed Army Institute of Research participated in clinical trials and FDA approval for the sulfadoxine-pyrimethamine combination (Fansidar; Hoffman-LaRoche) for malaria prevention. After approval was granted in other countries, Fansidar was approved by the FDA in 1983. Although Fansidar is still used as malaria treatment in some countries that lack alternative antimalarial drugs [20], use of Fansidar is limited because of infrequent but serious adverse reactions, including Stevens-Johnson syndrome, and the widespread emergence of Fansidar-resistant malaria strains.

**MEFLOQUINE**

US scientists discovered the prototype drug for mefloquine, SN 10275, during World War II. The first-generation synthetic quinoline methanols caused unacceptable phototoxicity. Mefloquine (WR 149240) was developed by the Division of Experimental Therapeutics at Walter Reed Army Institute of Research in the late 1960s in collaboration with the World Health Organization Special Programme for Training and Research in Tropical Diseases (WHO/TDR) and Hoffman-LaRoche. Meflo-
The antibiotic doxycycline (Vibramycin, manufactured by Pfizer) has slow-acting antimalarial properties via binding to malaria ribosomes, thereby inhibiting protein synthesis. Walter Reed Army Institute of Research conducted Phase 2 challenge trials and clinical trials with doxycycline in Thailand, and FDA approval was granted to Pfizer in December 1992 for its use as prophylaxis for *P. falciparum* and *P. vivax* malaria [24, 25]. From June 1992 to November 1993, >2000 Dutch military personnel serving in Cambodia used doxycycline for malaria prophylaxis; only 59 developed malaria.

Although doxycycline is a valuable antimalarial, and although it has an additive effect with quinine for treatment of *P. falciparum*, it requires daily administration and may cause phototoxicity, gastrointestinal discomfort, and diarrhea, all of which reduce compliance with the regimen. Lodging of doxycycline tablets in the esophagus may cause serious chemical damage. Furthermore, doxycycline cannot be given to pregnant women or children because of tooth discoloration in developing teeth.

**ERYTHROMYCIN AND AZITHROMYCIN**

The antibacterial drugs erythromycin and azithromycin have antimalarial effects related to inhibition of mitochondrial protein synthesis [26]. Azithromycin’s excellent safety profile in children and pregnant women prompted antimalarial prophylaxis evaluations in Kenya, Indonesia, and Thailand by military investigators in the mid-1990s. Prophylaxis efficacy was excellent (99%) for *P. vivax* malaria, but for *P. falciparum* malaria, it ranged from 70% to 83%, even with daily dosing [27, 28]. The US military, in partnership with Pliva Pharmaceuticals, is seeking azithromycin analogues with improved and more-selective antimalarial activity and similar safety and pharmacokinetic characteristics. Combination of azithromycin with other antimalarial agents for malaria is also under investigation. Synergy is noted when azithromycin is combined with chloroquine [29].

**ATOVAQUONE-PROGUANIL**

Malarone (GlaxoSmithKline), approved by the FDA for prevention and treatment of *P. falciparum* malaria in 2000, is a combination of atovaquone (a substituted 2-hydroxynaphthoquinone) and proguanil. These drugs act against blood forms and early liver stages of malaria. The structure of atovaquone differs markedly from other antimalarials. Atovaquone is marketed in the United States as a single agent under the trade name Mepron (Glaxo Wellcome) for treatment of *Pneumocystis jiroveci* (previously named "*Pneumocystis carinii*”) pneumonia. Atovaquone inhibits the electron transport system of the malaria parasite at the level of the cytochrome *bc* complex, thereby blocking pyrimidine biosynthesis, which is essential for *Plasmodia* mitochondrial function (in contrast, mammalian cells are able to salvage pyrimidines). Atovaquone also causes collapse of the mitochondrial membrane potential in *P. falciparum*. Proguanil (Paludrine; ICI), which interferes with folic acid synthesis via binding to dihydrofolate reductase (much like pyrimethamine), was identified by British scientists Curd, Davy, and Rose in 1945 [30] as a drug with low toxicity and high activity against avian malaria. Proguanil was approved by the FDA in 1948 as an antimalarial agent, but it was not widely

**HALOFANTRINE**

Halofantrine (WR 171669) was developed at Walter Reed Army Institute of Research in collaboration with SmithKline Beecham and the WHO/TDR beginning in the late 1960s and early 1970s as a backup drug to mefloquine to treat chloroquine-resistant *P. falciparum* malaria. Halofantrine was created by replacing the quinoline moiety of the quinoline methanols (quinine-type compounds) with other aromatic groups, to form the aryl (amino) carbinols. Of this class of compounds, halofantrine, a 9-phenanthrenemethanol, is the most potent. Commercial development began in the 1980s, and the drug (Halasan) was approved by the FDA in July 1992.

Although halofantrine is still used for treatment of *P. falciparum* malaria outside of the United States in areas where other antimalarial drugs are unavailable, use of halofantrine is limited by its short half-life (1–2 days), slow and variable absorption, and adverse effects (especially potentially fatal cardiotoxicity related to prolonged electrocardiographic QT intervals and embryotoxicity). Its main metabolite appears to be a less toxic and equally efficacious antimalarial, but this was not further developed [23].

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used, and marketing its use as a single drug ended in the United States in the 1970s. Proguanil is still used overseas as an antimalarial, especially in combination with chloroquine (malaria parasite resistance to proguanil monotherapy has been recognized since the 1950s). However, the primary role of proguanil in Malarone may not be as an antifolate. Although the mechanism is unclear, there is profound antimalarial synergy when atovaquone and proguanil are combined.

Development of drug combination strategies, dose-ranging preclinical and clinical studies, and pivotal efficacy trials were organized by Walter Reed Army Institute of Research in partnership with GlaxoSmithKline. Atovaquone was consistently effective in clearing parasitemia in uncomplicated *Plasmodium falciparum* malaria, but recrudescence rates up to 25% precluded further development as monotherapy. After studies at the Armed Forces Research Institute of Medical Sciences confirmed synergy between atovaquone and proguanil, coadministration trials conducted at various sites, including Walter Reed Army Institute of Research laboratories in Brazil and Kenya, demonstrated 99% efficacy for treatment of uncomplicated multidrug-resistant malaria and 98% efficacy for prophylaxis in placebo-controlled trials. Additional prophylaxis studies were completed by the Naval Medical Research Unit-2 in Jakarta, Indonesia.

Although Malarone has few adverse effects other than mild gastrointestinal intolerance, experience with this expensive drug is limited. Rapid development of drug resistance related to mutations mostly arising in the cytochrome b gene of *P. falciparum* has been noted when Malarone has been used as malaria monotherapy [31].

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