Nobel for Artemisinin
Curcumin is Waiting for Ages

G Padmanaban

The award of Nobel Prize to Madam Youyou TU from China for the discovery of artemisinin and its derivatives (ART) that has led to the cure of millions of malaria patients has rekindled the interest in natural products as a source of potent drugs. Curcumin from turmeric has a huge potential to cure several diseases and this article provides the basis for its use in combination with ART to cure malaria and many other diseases.

Interestingly, the drug for malaria was discovered by the order of Mao Zedong, considered as the supreme leader of China at one time. He wanted to save the North Vietnamese soldiers, fighting against the US in the forests in the 1960s, from succumbing to chloroquine-resistant malaria. Madam Tu who had graduated from the Department of Pharmacy at Beijing Medical University and was working at the China Academy of Traditional Chinese Medicine, led a group that screened 200 recipes with Chinese traditional herbs and 380 extracts from the herbs were tested on P. berghei-infected mice and P. cynomolgy-infected monkeys. Finally success was achieved with extracts from Artemisia annua. She established the safety of the extracts in humans, including herself, and found them to be very effective in curing P. falciparum and P. vivax-infected malaria patients. Artemisinin was isolated as the active ingredient and characterized as a sesquiterpene lactone. Several derivatives have been prepared for clinical use (Figure 1).

Madam Tu was not a well-known scientist before she was awarded the Nobel Prize. She was not even a Fellow of the Chinese Academy of Sciences, but doggedly pursued her goals. Her results were published in Chinese journals [1], before her work was recognized outside [2]. She richly deserves the Nobel Prize.

Keywords
Artemisinin, turmeric, curcumin, malaria, plasmodium.
Although malaria incidence has been brought down by 50% through mosquito control and protection measures and due to effective treatment with artemisinin derivatives (ART), it still infects 250 million people around the world and kills 600,000 people every year. ART remain the only hope at this time to treat malaria, since the malaria parasite has become resistant to frontline drugs such as chloroquine and antifolates. Among the species of *Plasmodium* infecting the human, *P. falciparum* and *P. vivax* account for 95% of malaria in the world, although species such as *P. knowlesi* are also coming into prominence.

To prevent resistance development to ART, WHO has warned against the use of monotherapy and emphasized the use of ART-based combination therapy. Thus, artemether-lumefantrine (Coartem), dihydroartemisinin-piperaquine (Euartesim), etc., are made available at subsidized costs in Africa, which is the epicenter of global malaria, reporting almost 90% of the deaths. But, unfortunately, resistance is being reported to ART-based therapy in Southeast Asian countries and seems to be spreading [3]. It is in this context that our own study with ART-Curcumin combination therapy to treat experimental malaria has become relevant.

1 Malaria in animal models.
How I Got into Malaria Research

I have a story to tell. I started working with the malaria parasite in the 1980s. Earlier, I was working on fundamental aspects of eukaryotic gene transcription and its regulation. In 1981–82, Astra-Sweden came to me for some reason to help them set up a research centre in Bangalore, close to the Indian Institute of Science (IISc), Bangalore, and devoted to infectious diseases. I was excited and drew a blueprint. As a professor at the Department of Biochemistry, IISc, I could convince close to half-a-dozen of our own students who were finishing their PhD to do something different from rushing to go abroad. I was successful and designed four projects, namely on tuberculosis, malaria, diarrheal diseases and cysticercosis. We started research in my own laboratory and Astra gave funds to build an entire new laboratory in the department. The then Directors, Dr S Ramaseshan and Dr C N R Rao were very supportive and thus was born Astra Research Centre (ARC)! Subsequently, ARC moved to a location close by (near the Sankey lake) and my interaction continued for a few more years. I had a joint student from ARC, who registered for PhD at IISc and started working with the malaria parasite.

Research on malaria parasite provided a link to my interest on heme, which we had earlier shown to be a multifunctional regulatory molecule in living cells, apart from being the prosthetic group of cytochromes and other hemoproteins. The life cycle of the malaria parasite consists of the liver, blood and mosquito stages (Box 1). In the blood stage, also known as the intra-erythrocytic stage, the parasite grows and develops in the red blood cell. It lyses the red cell and the released merozoites infect fresh ones. This process synchronises with high fever and chills as seen in malaria patients. The parasite acquires hemoglobin and other nutrients from the red cell. The globin part is degraded into amino acids, which the parasite uses for its own protein synthesis. At the same time, the parasite also acquires large amounts of heme, which is a toxic molecule. The parasite detoxifies monomeric heme by converting it into a microcrystalline aggregate, referred to as hemozoin. Drugs such as chloroquine and other  

2 A small molecule such as heme being part of cytochromes and hemoproteins.
Box 1. Life Cycle of the Malaria Parasite

When the anopheles female mosquito carrying the malaria parasites has a blood meal, sporozoites (2–200) from the salivary glands are released into blood through the skin. Quickly the parasites are lodged in the liver hepatocytes. These differentiate into schizonts and then into merozoites over 5.5 days (*P. falciparum*) and each liver cell releases thousands of merozoites to infect the erythrocytes. In the blood, the parasite undergoes asexual cycle through ring, trophozoite, schizont and merozoite stages. Each schizont can give rise to 16–18 merozoites that burst out of the red cell and start the next cycle with fresh cells. During the intraerythrocytic stage, some merozoites differentiate into male and female gametocytes that mature over 8–10 days through the process of gametogenesis. During a mosquito bite, the gametocytes enter into the mosquito gut and develop into gametes. Significant morphological changes take place during gametogenesis. Fertilization of female gametes by the male gametes gives rise to zygotes that give rise to ookinetes. Zygote and ookinite are the only diploid forms in *P. falciparum*. The ookinite traverses through the midgut epithelium and enters the basal lamina to give rise to oocysts. These undergo meiotic division over the next one to three weeks and give rise to multiple haploid immature sporozoites. These break through the oocyst cell wall into the haemolymph and develop into mature sporozoites lodged in the salivary glands and are ready for the next invasion.

Almost all the parasites in the blood stage are in the same stage of development giving rise to a synchronous cycle and this is dependent on the circadian rhythm of the host in terms of body temperature. The disease manifestation in terms of high fever and chills accompanies this cycle. In general infected red cells are killed by appropriate antimalarials. However, *P. falciparum* in particular, is capable of altering the cell surface proteins of the red cell that results in the binding of such cells to receptors on the endothelium membrane of organs and placenta. This is a major complication leading to human cerebral malaria, organ failure and stunted fetal growth.

quinolones as well as ART prevent this detoxification process by the parasite. This will lead to accumulation of monomeric heme that will kill the parasite. ART has also other sites of action. One of my interests continues to be to unravel the heme dynamics in the parasite in all stages of its growth.
Simultaneously, in another project we have been screening the antimalarial activity of natural compounds. This expedition led us to the investigation on curcumin as an antimalarial.

World of Curcumin

As I started reading about curcumin, its history and curative properties, it left me with wonder and awe. Turmeric contains curcuminoids generally in the range of 3–6% and curcumin is isolated from this herb. Turmeric is used extensively in Indian cooking and is known since Vedic times for its medicinal use. There are many Ayurvedic recipes containing turmeric with wide applications ranging from treatment of wounds, sprains, skin infections, lung congestion, scorpion sting, etc. It is also described in Charaka Samhita as a remedy for jaundice, hemorrhoids (piles), snake bites, conjunctivitis, skin blemishes, chicken pox, etc. Curcumin, diferuloyl methane (Figure 2), was isolated in 1815 from turmeric and it can be stated without exaggeration that there is no disease against which curcumin has not been tested and found to be effective. Curcumin has been tested in over 100 clinical trials, mostly cancers and other lifestyle diseases and in general found to have beneficial effects. But, the invariable conclusion is that more detailed studies are needed with larger randomized clinical trials [4]. Interestingly, curcumin is also reported to act on multiple molecular targets (Figure 3). These may also be the reasons as to why curcumin is not an approved drug even for a single ailment, although US-FDA considers it as GRAS (Generally Regarded As Safe) molecule. Pharma companies in general would like a drug molecule to cure a specific disease with a specific site of action. It is difficult to take a drug forward, if it is claimed to act on multiple host targets and at the same considered as non-toxic. On top of all this, the bioavailability of curcumin is poor and it is metabolized very fast. I felt that there is something unique about this molecule in terms of its mechanism of action that needs to be established and taken forward. This has led me
to suggest that to start with curcumin should be tested as an adjunct drug (in addition to standard therapy) against infectious diseases [5]. The basis for this suggestion and implications would become clear once I describe our results on the curative effects of curcumin in experimental malaria.

Curcumin Prevents Malaria Parasite Recrudescence

Maximum mortality of malaria patients is due to *P. falciparum* infection. When *P. falciparum*-infected malaria patients are treated with drugs, about 5–10% return after some weeks with fever. This phenomenon is known as recrudescence and can be due to several reasons. One reason is the reappearance of the same parasite that can potentially evolve to cause severe malaria. Initially, we found that curcumin has antimalarial action in a culture of *P. falciparum* with an IC$_{50}$ (50% inhibitory concentration) of 5–10 µM. Oral administration of curcumin was also found to temporarily inhibit parasite (*P. berghei*) growth in Swiss mice, delaying death by a few days, while infected, untreated animals died between 6–8 days. This can only be considered as a modest effect and we wanted to examine the potential of ART-curcumin (AC) combination to prevent parasite recrudescence.
We established a parasite recrudescence model in mice by giving a single injection of a sub-optimal dose of \( \alpha,\beta \)-arteether (AE). We found that a single injection of 750 \( \mu \)g of AE to mice after three days of infection with \( P.\)berghei cleared the parasites in blood and protected the animals for about 20 days. However, the parasites started appearing in blood after 20 days, eventually killing the animals between 26–34 days. However, if the animals had received three oral doses of curcumin at the time of AE injection, parasite recrudescence was completely prevented. There was almost 100% protection of the animals against mortality (Figure 4). This was an exciting observation and we studied the

**Figure 4.** Effect of curcumin in a malaria parasite recrudescence model in mice. Swiss mice infected with \( P.\) berghei were given a single injection of AE after 72 hr of infection. (A) Parasite-infected animals died between 6–8 days. Depending on the concentration given, the death of animals was delayed by 4–25 days. Animals receiving a single injection of 750 \( \mu \)g of AE died between 32-34 days. There was 100% protection against mortality in animals receiving three oral doses of curcumin (5mg per animal/dose) along with the single injection of 750 \( \mu \)g of AE. (Adapted from D N Nandakumar *et al*, Curcumin-artemisinin combination therapy for malaria, *Antimicrobial Agents and Chemotherapy*, Vol.50, pp.1859–1860, 2006.) (B) Parasite was cleared in blood soon after a single injection of 750 \( \mu \)g of AE. But, the parasites started appearing after 21 days and reached around 60% when the animals died between 26–34 days. However, no parasite could be seen in the blood smear of animals receiving three oral doses of curcumin along with a single injection of 750 \( \mu \)g of AE. (dpi: days post infection).

Adapted from P G Vathsala, PhD thesis entitled ‘Identification of chloroquine resistant haplotypes of *Plasmodium falciparum* in India and development of new antimalarial combinations’, 2006.
mechanisms involved in detail [6]. We found that parasites lodged in liver and spleen were not cleared by AE or AC treatment, but parasite recrudescence in blood was completely prevented in AC treatment. These studies led to the conclusion that curcumin in combination with AE has short-term and long-term actions. In the short-term it potentiates the production of reactive oxygen species (ROS) to kill the parasite. In the long-term, it inhibits inflammatory response and stimulates anti-inflammatory response leading to anti-parasite antibody production, whenever the animal is challenged with fresh infection (Figure 5).

These results have also laid the foundation for understanding the unique mechanism of action of curcumin. Almost all the studies with curcumin on cancer cell lines, for example, indicate that the IC\textsubscript{50} for curcumin is in the micromolar (usually 10–20 µM) range as we also found to be the case with \textit{P. falciparum} culture. Curcumin inhibits all the targets described in literature [7] in this concentration range. However, \textit{in vivo} bioavailability of curcumin is very low, of the order of 10–20 nM in blood. This is almost a 1000-fold less concentration in blood than that required to directly kill the parasite in culture. Curcumin is also metabolized very fast and thus has a transient existence of a few hours. At the same time, we have shown that curcumin prevents parasite recrudescence even after a month, when both AE and curcumin have
been eliminated from the system long back. Curcumin in the absence of AE does not have a long-term effect. Thus, we have proposed that AE kills the parasite to start with, generating parasite antigens. Curcumin, during its transient presence, is able to create immune memory to the parasite antigens. These memory cells will come into play whenever there is fresh infection and provide protection. This is the basis for the proposal to use curcumin as an adjunct drug in other infectious diseases and perhaps cancer treatment as well [5].

**Curcumin Prevents Experimental Cerebral Malaria**

Our next target was to investigate whether curcumin can prevent cerebral malaria, since mortality in Human Cerebral Malaria (HCM) is high (15–25%), despite intravenous infusion with artesunate and an adjunct drug is urgently needed. We established the Experimental Cerebral Malaria (ECM) model using C57BL mice infected with *P. berghei* ANKA strain. More than 80% of the animals showed typical cerebral malaria symptoms such as convulsion, hind leg paralysis, ruffled hair, etc., finally leading to coma and death between 8–12 days. Interestingly, we found that three oral doses of curcumin given 72 hr after infection, but before the onset of symptoms, completely prevented cerebral malaria. But, the animals died after 20 days due to parasite build-up in blood causing anemia. Curcumin was also able to inhibit breakdown of blood-brain barrier, which is a hallmark of cerebral malaria. Once again study of a large number of cytokines, chemokines and their receptors in terms of mRNA levels in brain and some of the antigen levels in the serum clearly indicated that curcumin prevented inflammatory responses and promoted anti-inflammatory responses. Curcumin treatment also inhibited NF *kappa* B phosphorylation. NF *kappa* B is a molecular conduit leading to activation of genes involved in inflammatory response. A major feature of HCM is the sequestration of T lymphocytes (CD8+ T cells) and parasite-infected RBCs (pRBCs) in the brain. We could show that curcumin could inhibit the sequestration of both CD8+ T cells and pRBCs in the mouse brain.
In order to prevent the slow build-up of parasites in blood eventually leading to death due to anemia and to offer a cure after the onset of cerebral malaria symptoms, we used the AC combination therapy and showed that it can completely cure the animals, preventing parasite accumulation and mortality. We have proposed a model [8] to explain the mechanism of action of curcumin to prevent and cure cerebral malaria in combination with ART (Figure 6).

**Figure 6.** A scheme to explain the mechanism of action of curcumin to prevent Experimental Cerebral Malaria (ECM). When C57BL mice are infected with *P. berghei* ANKA strain, they show cerebral malaria symptoms and die between 8–12 days. Three oral doses of curcumin on days 3, 4 and 5, before the onset of symptoms, prevent the onset of the symptoms and delay death by almost 20 days. Curcumin alone inhibits NF κB phosphorylation, preventing the inflammatory cascade and pRBC sequestration in brain. But, the animals eventually die due to parasite build-up in blood and anemia. However, if the animals are treated with AE and curcumin, even after the onset of symptoms, the parasites are cleared and the animals are completely protected against mortality. Thus, while curcumin counteracts the inflammatory response, AE prevents parasite build-up and thus making this combination as a potential therapy to prevent and cure human cerebral malaria. (Adapted from C Dende *et al.*, Simultaneously targeting inflammatory response and parasite sequestration in brain to treat experimental cerebral malaria, *Sci. Rep.*, Vol.5, p.e12671, 2015.)
Future Perspective

Finally, it is not really a question of curcumin leading to a Nobel Prize. The title given for this article is to enthuse students and researchers to unravel the mystery of the diverse potential of curcumin to cure a large number of diseases, understand its molecular mechanism of action and take it to the logical conclusion of its use in the clinic. I believe, our study with the malaria parasite has laid the basis. A probable explanation has emerged from our studies with the malaria parasite that it can create immune memory to the antigens generated by the primary antimalarial drug (AE). Curcumin has the potential to decrease the dose of ART used in chemotherapy and to prevent emergence of resistance to the primary drug, besides potentiating its action. Thus, curcumin, while it can provide a longer lease of life to Madam TU’s molecule, may also have a similar synergistic action with many other drugs.

In a broader context, curcumin as an adjunct drug would be effective as an immunomodulator of the host response rather than by its direct killing effect of the parasite or the cancer cell. Efforts are underway in many laboratories to increase bioavailability of curcumin with the use of nano- and other formulations. These efforts could improve the efficacy of curcumin to a modest extent. It may also be not desirable to increase the in vivo concentration of curcumin beyond a threshold level. Given its propensity to inhibit a large number of molecular targets, it may actually prove to be harmful. We are trying hard to mount a human clinical trial with curcumin as an adjunct drug in uncomplicated and cerebral malaria. While we are very optimistic, we need to remember that men and mice are not the same and we need to keep our fingers crossed till we actually conduct the clinical trials and analyse the data! If eventually curcumin emerges as a universal adjunct drug or at least to cure specific diseases of major concern, it will be a vindication of the sound experience-based evolution of the ancient system of traditional Indian medicine. We can all be proud that we have been able to understand the wisdom of our ancestors in modern scientific terms.
Acknowledgement

Research in my laboratory was supported by grants from the Department of Biotechnology and Fellowship from the Indian National science Academy, New Delhi. I want to thank Dr Arun Nagaraj for helping me with the figures provided in this article.

Suggested Reading


