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A Postulated Mechanism of the Antimalarial Effect of Free Radicals Generated by Artemisinin on *Plasmodium falciparum*

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Artemisinin and its derivatives, a class of antimalarial drugs, were first isolated from Artemisia annua. Artemisinin can alter the pH of the malaria parasite's digestive vacuole from acidic to alkaline, leading to parasite death. However, the precise mechanism of artemisinin action in changing the digestive vacuole pH has not yet been confirmed. Previous studies reported that artemisinin and its derivatives could kill the parasites through the generation of oxidative stress by the free radicals they generate. This review aims to provide a better understanding of the possible mechanism of action of artemisinin, focusing on the antimalarial activity caused by the generated free radicals through the induction of mutation in the genes that encode the proton pump of the Plasmodium falciparum digestive vacuole.

Keywords: Artemisinin; Free radicals; Proton pump; Plasmodium falciparum; V-type H+-ATPase; VMA gene.

Malaria is a life-threatening disease caused by parasites of the genus *Plasmodium* that is transmitted to humans by female mosquitoes of the genus *Anopheles*[1]. The infection has been identified as one of the major causes of morbidity and mortality globally[2]. Efforts have been made to identify effective antimalarial drugs over decades of years; however, drug-related problems have emerged in parallel with these discoveries[3,4].

Multiple types of effective antimalarial drugs have since been introduced as alternatives to the original treatments, such as artemisinin. Artemisinin, a sesquiterpene lactone, was first isolated from the Chinese plant, *Artemisia annua* in 1970[5]. The 1,2,4-trioxane system contains an endoperoxide bridge, the active pharmacophore, which plays an important role in the antimalarial activity of artemisinin[6]. Although it has been

considered one of the most important drugs for the treatment of malaria, the exact mechanism of action remains controversial[7].

MATERIALS AND METHODS

Methods

We conducted an extensive review of the literature on the antimalarial effects and mechanisms of action of artemisinin, focusing on *in vitro* studies conducted to evaluate the pH alteration of the *P. falciparum* digestive vacuole after treatment with this compound.

RESULTS AND DISCUSSION

Previous studies reported that antimalarial drugs, such as artemisinin and its derivatives consist of free radicals that are a source of oxidation, which may cause parasite death[8]. Artemisinin produces reactive oxygen species (ROS) in the digestive vacuole through the activation of the endoperoxide bridge, and free Fe^{2+} can increase the generation of ROS via the Fenton process. Thus, it has been reported that artemisinin-activated ROS may lead to parasite death by reducing the ability of the parasite's antioxidant defence system to remove free radicals[9].

On the other hand, it has been reported that artemisinin can alter the pH of the *P. falciparum* digestive vacuole from acidic to alkaline[10] (Figure 1). Thus, the protease enzyme that functions only within the digestive vacuole with a pH ranging from 3.7–6.5[11], and which is responsible for the digestion of haemoglobin to release the amino acids as a nutrient source for the parasite, is inhibited, leading to the parasite death[12]. However, the precise mechanism behind this phenomenon remains controversial[13].

Permeabilized resealed erythrocytes infected with mid trophozoite stage parasites containing FITC-dextran, a ratiometric pH indicator, treated with 15, 30 (sub-lethal concentrations), 60 (a concentration near the $IC_{50\text{-}4\ hours}$), 1000 nM (a positive control), and concanamycin A (a standard proton pump inhibitor), confirming the induction of acid-base transition. A non-treated condition was used as a negative control. The data are expressed as mean ± SEM from three independent experiments done in triplicates. The bar graph was generated using GraphPad Prism (version 7). Modified from Ibrahim (2020)[10].

We hypothesized that the alteration of the digestive vacuole pH from acidic to alkaline after treatment with artemisinin might result from the induction of mutation by the free radicals, such as superoxide anion (O_2^{-}) generated from this class of antimalarial drugs in the gene (*VMA*) encoding the proton pump (V-type H^+-ATPase) that is responsible for providing an acidic environment for the parasite's digestive vacuole, as well as for other microorganisms, including yeast[14,15,21].

Previous studies have reported that the biochemical and genetic properties of yeast V-ATPases are similar to those of other eukaryotic cells; these sharing features encourage the use of

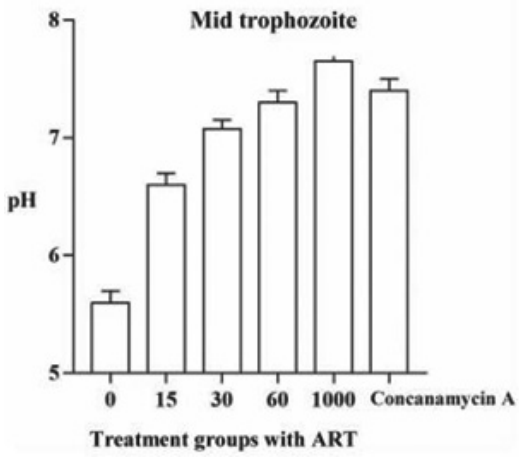

Fig. 1. Artemisinin altered the pH of the digestive vacuole of the mid trophozoite stage parasites

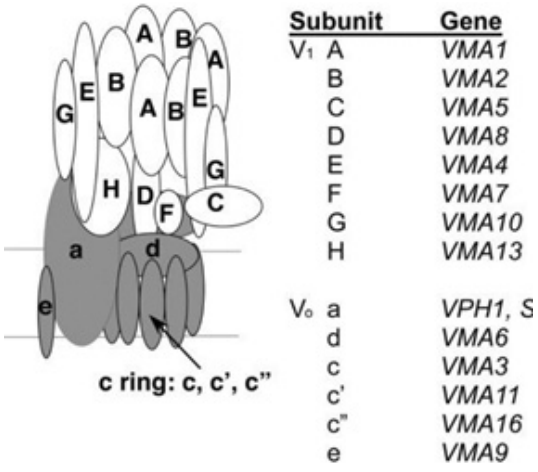

Fig. 2. Yeast V-ATPase structure model and subunit gene designations

yeast V-ATPase as a model for the study of the V-ATPases located at the *P. falciparum* digestive vacuole's membrane[16].

Yeast V-ATPase consists of a cytosolic V_1 domain, which catalyzes the ATP hydrolysis and the integral membrane V_o domain, which forms the transmembrane proton pore. V-ATPases are responsible for controlling the acidification of yeast and other microorganisms via reassembling and disassembling the V-ATPase complex [17] (Figure 2).

The V_1 domain consists of eight subunits, labeled as A, B, C, D, E, F, G, and H. Whereas the V_0 domain consists of six subunits labeled as a, c, c', c", d, and e. Each of these subunits is encoded by the following genes; *VMA1*, *VMA2*, *VMA3*, *VMA4*, *VMA5*, *VMA6*, *VMA7*, *VMA8*, *VMA9*, *VMA10*, *VMA11*, *VMA13*, and *VMA16*[19] (Figure 2).

The free radicals have the capability of inducing oxidative damage that leads to a cellular DNA mutation [20]. Once a mutation occurs in the genes encoding the yeast V-ATPase, the vacuolar acidification will be lost[21]. This postulates that a mutation can also be induced in the gene encoding the V-type H^+- ATPase of the *P. falciparum* digestive vacuole via free radicals generated from artemisinin, ultimately leading to parasite death.

CONCLUSION

In summary, the present review provides a better understanding of the antimalarial activity of artemisinin and illustrates how artemisinin may alter the pH of the *P. falciparum* digestive vacuole through the induction of mutation in the gene that encodes the proton pump, V-type H^+-ATPase of this organelle through the subsequently generated free radicals.

However, the lack of experimental evidence might have somewhat limited the demonstration of a true cause-and-effect relationship in this study. Therefore, further studies are needed to identify the specific genes that encode the proton pump V-type H+-ATPase of the parasite digestive vacuole, as well as to confirm the induction of mutation in those genes by the free radicals generated from artemisinin.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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