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In Vitro and in Vivo Antimalarial Activity of Linolenic and Linoleic Acids and their Methyl Esters

Paula Melariri[1], William Campbell[1], Paschal Etusim[2] and Peter Smith[1]

[1] Division of Pharmacology, Department of Medicine, University of Cape Town Medical School K45, Old Main building Groote Schuur Hospital, Observatory 7925 South Africa

[2]Abia State University Uturu, PMB 2000 Uturu, Abia State Nigeria

Correspondence should be addressed to Paula Melariri, paulamelariri@gmail.com

Abstract

Essential fatty acids (EFA) are a group of unsaturated fatty acids which are not produced in humans, but are necessary for the proper functioning of the human body. It is mostly found in vegetable oils, seeds and nuts. They have shown diverse medicinal properties and potential beneficial effects on diseases such as cancer, insulin resistance, skin permeability, cardiovascular disease and depression. Fatty acids have the potential to inhibit the fatty acid biosynthetic machinery of *Plasmodium falciparum* parasite. In the present work, *in vitro* antiplasmodial activity of linoleic and linolenic acids using PLDH assay, against D10 and Dd2 strains of the parasite was <10 μg/ml. Linolenic and linoleic acids inhibited parasites growth by 70% and 64% respectively, against *P.berghei* using the 4-day suppressive test. The two compounds when used in combination inhibited the parasites by 96% on day 4 of treatment. No significant difference was observed between the free acids and their methyl esters.

Keywords: *In vitro; In vivo;* Antiplasmodial; linolenic, linoleic, Methyl ester.

1. INTRODUCTION

Malaria is caused by an apicomplexan parasite in the genus *Plasmodium.* Four species of the genus cause human malaria- *P.vivax, P. ovale,* P.*malariae* and *P falciparum. P. falciparum* is the most deadly malaria parasite and has been found to contain an apicoplast, which is an organelle originally from cyanobacterium, and has undergone a secondary endosymbiotic process and possesses four membranes [1]. The apicoplast is an indispensable organelle for the parasite as many vital metabolic processes such as, fatty acid biosynthesis which is necessary for the growth of the parasite take place there. The parasite needs the fatty acid for the construction of cell membranes, as a source of energy, signal transduction, protein acylation, growth, differentiation and homeostasis. Interestingly, the *Plasmodium falciparum* parasite uses type II fatty acid synthase while humans make use of type I fatty acid synthase [2]. This indicates that the parasite's fatty acid synthase system could be selectively targeted without harming the host. The apicoplast is also essential for the biosynthesis of isoprenoids and this is achieved by the mevalonate-independent 1-deoxy-D-xylulose 5- phosphate (DOXP) pathway. The apicoplast houses the enzymes of the DOXP pathway. This pathway is required for malaria parasite growth, but not present in humans.

In recent years, there has been an increase in the role of specific dietary fatty acids and their medicinal properties. The polyunsaturated fatty acids (PUFA) potential health-related benefits to be explored such as improving heart disease related outcomes, decreasing tumour growth and metastasis, and regulation of insulin sensitivity. Moreso, there is a growing realisation that fatty acids have the potential to inhibit the fatty acid biosynthetic machinery of *Plasmodium falciparum* parasite [2]. Fatty acids have shown antimalarial, antimycobacterial and antifungal properties [2]. Essential fatty acids (EFA)m were reported to be of benefit in the prevention and management of coronary heart disease, stroke, diabetes mellitus, hypertension, cancer, depression schizophrenia, Alzhemer's disease, and collagen vascular disease [3]. Previous work reported on the antimalarial properties of n-3 and n-6 polyunsaturated fatty acids [4]. Polyunsaturated fatty acids especially the essential fatty acids have medicinal properties.

Polyunsaturated fatty acids are hydrocarbon chains with ≥2 double bonds. These hydrocarbons can be classified as n-6 or n-3 owing to the location of the first double bond relative to the methyl terminus. Essential fatty acids (EFA) are a group of unsaturated fatty acids which are not produced in humans, can be obtained in diet and are necessary for the proper functioning of the human body [5,6]. The two main families of EFAs are the ω-6 and the ω-3 groups. Linolenic and linoleic acids are essential fatty acids which belong to the omega 3 and omega 6 fatty acids respectively. These essential fatty acids have recently drawn attention and are recently marketed as health supplements due to the health

benefits associated with them [7,8]. Essential fatty acid deficiency (EFA) has been reported to result in abnormal keratinisation and permeability barrier function in rodents [9]. Furthermore, EFA deficiency leads to growth retardation, infertility, skin and kidney degeneration [10], capillary fragility, change in the function of the mitochondria as well as abrupt changes in fatty acid composition of lipids. The conjugated forms of these essential fatty acids have also shown promising biological activities. They are conjugated fatty acids due to the geometric position and configuration of their double bonds. Conjugated linoleic acids (CLA) are octadecadienoic acids with two conjugated double bonds. CLA have been shown to suppress human tumor [11] and were also recorded to reduce metastasis of cancers to lung tissue [12]. Other health potentials of CLA are antiathergenic, antidiabetogenic and immune modulating properties [13]. They are the only conjugated fatty acids that can be prepared in large quantities from natural sources [14]. Conjugated linolenic acids (CLnA) are octadecatrienoic acids with three conjugated double bonds.

Currently no effective vaccine against malaria is available but there are several candidates in development. Attempts have been made to introduce gamma radiation attenuated sporozoites (RAS) and genetically attenuated sporozoites (GAS). These findings with its promising potentials for vaccine development raised concerns on safety since none is 100% efficacious, such as the possibility of breakthrough infections [15,16,17] especially in immunocompromised individuals. Thus chemotherapy will remain the cornerstone of the multifaceted approach in malaria control, elimination and eradication in combination with available tools such as insecticide treated bed-nets and indoor residual spraying. Chloroquine (CQ) was used as a first line treatment of malaria types for many decades, but development of drug resistance by the parasite led to therapeutic failure [18]. The replacement of CQ with Sulphadoxine-Pyrimethamine (SP) was short lived due rapid selection of resistant parasites [19, 20]. This scenario had led to the use of a second line drug of choice -quinine (QN). QN acts in the erythrocytic phase against all types of *Plasmodium*. Although QN has recorded promising activity against chloroquine-resistant *falciparum* malaria, its use is limited by sits narrow therapeutic index, cardiotoxicity and the development of cinchonism syndrome [21], which is often characterised by neurological, cardiovascular and gastrointestinal toxicity as well as hypoglycaemia and hypersensitivity reactions. Primaquine which is a potent prophylactic drug against the liver stage of the disease has been shown to be toxic in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. The therapeutic effectiveness of artemisinins which recently have been the drug of choice is limited by a number of factors such as short half-life, neurotoxicity, and low solubility which affects its bioavailability [22, 23]. The derivatives of artemisinin eg artesunate and dihydroartemisinin have also recorded poor bioavailability [22]. Moreso, drug failure in candidates treated with artemisinin regimen has been recorded in some regions. A decrease in the sensitivity to artemisinin has now been confirmed in Cambodia [24,25, 26].

Pharmaceutical companies have lagged in the discovery of drugs for poverty related diseases due to the cost of the R&D, the market risk involved and the time-consuming nature of this field. Thus for drug discovery and development for a disease like malaria, where low returns can be expected, new or old therapeutic agents with new mechanism of action need to be explored. The challenges posed by toxicity, resistance to current treatment and the difficulties encountered with antimalarial vaccines or the production of new therapeutic agents emphasize the urgent need for the development of drugs with different mechanism of action for malaria therapy. The medicinal properties of essential fatty acids have recently drawn attention, moreso, the antiplasamodial properties of this group of fatty acids are yet to be fully explored. It is therefore the aim of this study, to investigate the in vitro and *in vivo* activities of linolenic and linoleic acids (Sigma) using Plasmodium parasites.

2. MATERIAL AND METHODS

2.1 Animals and diet:

This study was approved by the Animal Research Ethics Committee of the University of Cape Town, South Africa in 2007. All animal procedures were carried out in accordance with the suggested ethical guidelines for care of laboratory animals by the Animal Care and Use Committee of the University of Cape Town, as adapted from the recommendations of the Medical Research Council of South Africa [27]. The test animals were wild strains of C57 BL6 mice. Mice were obtained from the animal unit of the University of Cape Town (South Africa) at the ages between 7-10 weeks old. They were housed in standard cages in groups of five and placed on a pelleted custom diet. They were maintained under conventional conditions with controlled temperature (22±4ºC) and illumination (12h; 6:00 am to 6:00 pm) and had free access to standard diet and water *ad libitum*.

2.2 Parasite strain for in vitro experiment:

The chloroquine sensitive strain (D10) and the chloroquine resistant strain (Dd2) were used for this experiment. The sensitive strain was donated by Dr A. Cowman, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia while the resistant strain was derived from Indochina. The asexual erythrocytic stages of these parasites were maintained in a continuous culture using the method of Trager and Jensen [28].

2.3 Parasite strain for in vivo experiment:

The parasites used for this experiment were of the cryopreserved *P. berghei* (ANKA) strain. This parasite strain was donated by the Swiss Tropical Institute, Basel, Switzerland. Parasite stock was preserved in liquid nitrogen at -80ºC in the Division of Clinical Pharmacology, University of Cape Town. Parasite stock was

sustained by serial passage of blood from infected mice to uninfected mice. Parasitemia was monitored regularly. At a desired parasitemia, the mice were bled and euthanized. Blood samples collected were frozen in cryotubes and stored in liquid nitrogen at -80ºC.

2.4 Acute oral toxicity study of crude extracts:

Acute oral toxicity testing of drugs in mice was investigated at a dose of 100mg/kg for 4 days. Fatty acids and their methyl ester (FAME) were prepared according to the method of Kumaratilake *et al*., [4]. The dosage was administered orally. The test animals were monitored for 4 weeks to ascertain if there were any adverse events.

2.5 Sample preparation:

Linoleic and linolenic acids (Sigma) were used for the *in vitro and in vivo* experiments. The very lipophilic nature of these compounds meant that they were not readily soluble in an aqueous environment. Due to the lipophilic nature of the purified fatty acids, DL-α- Dipalmitoylphosphatidylcholine (DPPC) has been used as a vehicle to deliver these unsaturated hydrocarbons in *in vitro* and *in vivo* antimalarial assay [4]. DPPC alone showed no activity against plasmodium parasites [4]. Linolenic and linoleic acids were formulated in DPPC micelles according to Kumaratilake *et al*., [4]. Briefly, lipids were dissolved in chloroform (10-50 mg/5 ml). A mixture of fatty acids and DPPC micelles were prepared by adding DPPC at four-fold the amount of the fatty acid. Solvents were evaporated under nitrogen. A volume of 1 ml complete medium (RPMI 1640) was added to the mixture. The mixture was sonicated for two minutes and thereafter used for the assay.

2.6 In vitro antiplasmodial assays:

The antiplasmodial activity of fatty acids was evaluated against the chloroquine sensitive (CQS D10) and chloroquine resistant (CQR) strains of *P. falciparum* using the parasite lactate dehydrogenase (pLDH) assay as described by Makler *et al*., [29]. The IC50 recorded in this study is the mean of three independent experiments. The absorbance of each well was read using a microplate reader at 590 nm. The percentage parasite survival and the concentration that inhibits the growth of parasites by 50% were determined by measuring the conversion of NBT by *P. falciparum*. This was achieved by analyzing the readings from the microplate reader using Microsoft Excel® 2002, and the IC50 value which is the concentration at which the growth of the parasite was inhibited by 50% was determined using a non-linear dose response curve fitting analysis in Graph Pad Prism version 4.

2.7 In vivo antiplasmodial assays:

The four-day suppression test used in all *in vivo* antiplasmodial experiments in this study was as described by Peters *et al*. [30]. Animals were infected on day 0, (D_0) while treatment started 24 hours after infection (D_1). Malaria infection was

established in mice by the intraperitoneal (i.p) inoculation of 200 µl of $1x10^6$ parasitized cells/ml on the first day (D_0) of the experiment. Each mouse in the test group received 200µl of drug formulation 24 hours post infection. The dose was maintained for 4 consecutive days. Drugs were administered orally. To ascertain the parasitemia, on day 3 of experiment, thin blood smear were made and stained with 10% Giemsa in Phosphate buffer, pH 7.2 for 20 minutes. The slide was examined under microscope at 100_X. The Percentage parasitemia was determined by counting the parasitized red blood cells on at least 1,000 red blood cells in random fields of the Giemsa stained slide. The parasitemia of each group was determined and the standard deviation values taken as the mean value for the group. Each experiment had a positive control group and a negative control group. The positive control group received 200 µl of CQ (reference drug) at a dose of 10 mg/kg in Millipore water while the negative control group received 200 µl Millipore water only. The percentage growth inhibition was determined according to Tona *et al*., [31] as follows;

$$\% \text{ growth inhibition} = \frac{\text{Parasitemia of negative control} - \text{Parasitemia of test sample}}{\text{Parasitemia of negative control}} \times 100$$

2.8 Statistical analysis and data evaluation:

Parasitemia of all groups was monitored, and growth inhibition calculated, as shown earlier. The standard deviation values of parasitemia and weight were determined using the Microsoft Excel® 2002. The percentage parasitemia relative to the number of days post infection was evaluated using the Graph Pad Prism 4 version.

3. RESULTS

3.1 In vitro activity of linolenic and linoleic acids:

The *in vitro* antiplasmodial activity of linoleic and linolenic acids against both strains of the parasite was <10 µg/ml in this study (Table 3.1.1). The D10 strain used in the study was found to be CQ- sensitive with 50% inhibitory concentration (IC_{50}) value of 7.96±3.01ng/ml while the Dd2 strain showed IC50 value of 88.5±26.1ng/ml. Linolenic acid which has three double bonds, showed a higher antiplasmodial activity when compared to linoleic acid with two double bonds.

Table 3.1.1: *In vitro* activity of linolenic and linoleic acids against CQS D10 and the CQR Dd2 strains of *P. falciparum.*

Compound	IC50 D10 (µg/ml)	IC50 Dd2 (µg/ml)
Linolenic acid (Sigma)	4.12±0.25	5.04 ±1.24
Linoleic acid (Sigma)	7.80±0.67	7.56 ±0.92

3.2 *In vivo* schizontocidal experiment using the oral route of administration
Mice infected with *P. berghei* were dosed orally with linolenic and linoleic acids singly and in combination. In the single treatment each compound was administered at a dose of 100 mg/kg while in the combination treatment each compound was combined with the other at a dose of 50 mg/kg each, using the 4-day suppressive test of Peters *et al*. [30]. Linolenic acid significantly inhibited the growth of parasites compared to linoleic acid. The suppressive effect of the free acids when combined together (at a dose of 50 mg/kg each) proved to be more potent than the individual compounds alone (100 mg/kg). The percentage growth inhibition of the parasites by the free acids, singly and in combination is shown (Table 3.2.1).

Table 3.2.1: Percentage growth inhibition of compounds 1 and 2 individually and in combination on day 4 of treatment

Compound	%parasitemia	% growth inhibited
Millipore water	12%	Untreated negative control
Chloroquine	3.7%	69%
Linolenic free acid	3.6%	70%
Linoleic free acid	4.3%	64%
Linolenic + linoleic acids	0.5%	96%

Parasite growth inhibition was calculated using the formula below:

$$\% \text{ growth inhibition} = \frac{\text{Parasitemia of negative control} - \text{Parasitemia of test sample}}{\text{Parasitemia of negative control}} \times 100$$

The untreated control group, which is necessary to determine parasite growth inhibition, could not survive longer, thus day four post treatments was therefore chosen since it gives a good indication, of the rate of parasite suppression, induced by drug in a 4-day suppressive test.

3.3 In vivo schizontocidal experiment with fatty acid and their methyl esters using the oral route of administration:
To determine if the antiplasmodial activity of the methyl esters was different or similar when compared to the free acids, the linolenic and linoleic acid methyl esters were tested for antimalarial activity *in vitro* and *in vivo*. The methyl esters of linolenic and linoleic acids were purchased from Sigma (purity ≥ 99%). The *in vitro* activity was similar for the free acids and their methyl esters. Similarly, no significant difference was shown for the *in vivo* antiplasmodial activity of the

two free acids and their methyl esters using the oral administration (Figs. 3.3.1and 3.3.2). The dose response curves of the *in vivo* schizontocidal activity of the free acids and their methyl esters are shown below.

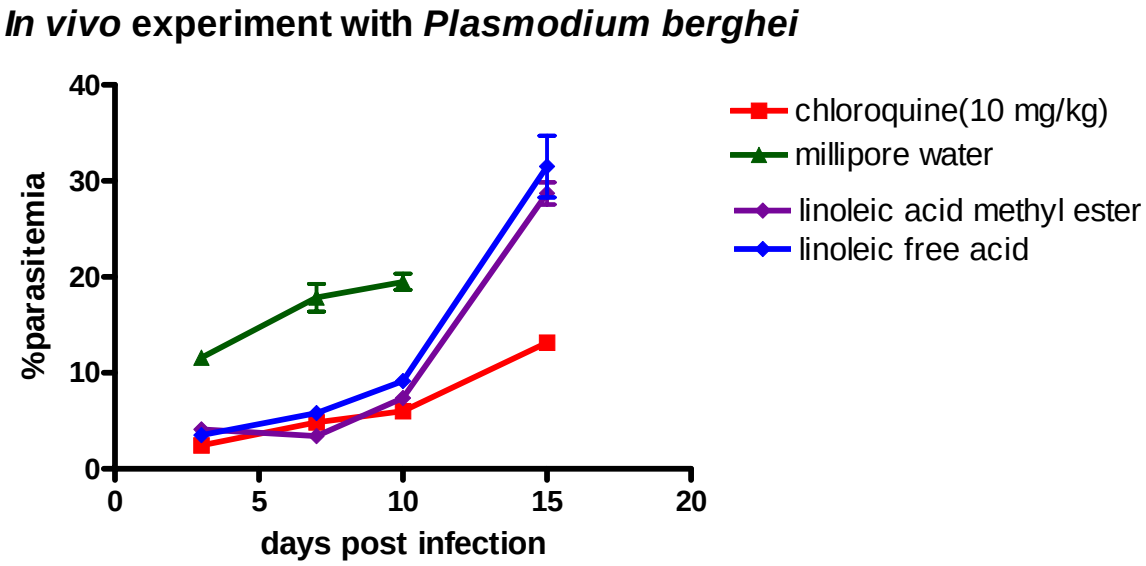

Fig 3.3.1: The *in vivo* activity of linoleic acid and the its methyl ester in a 4-day suppressive test using the oral route of administration.

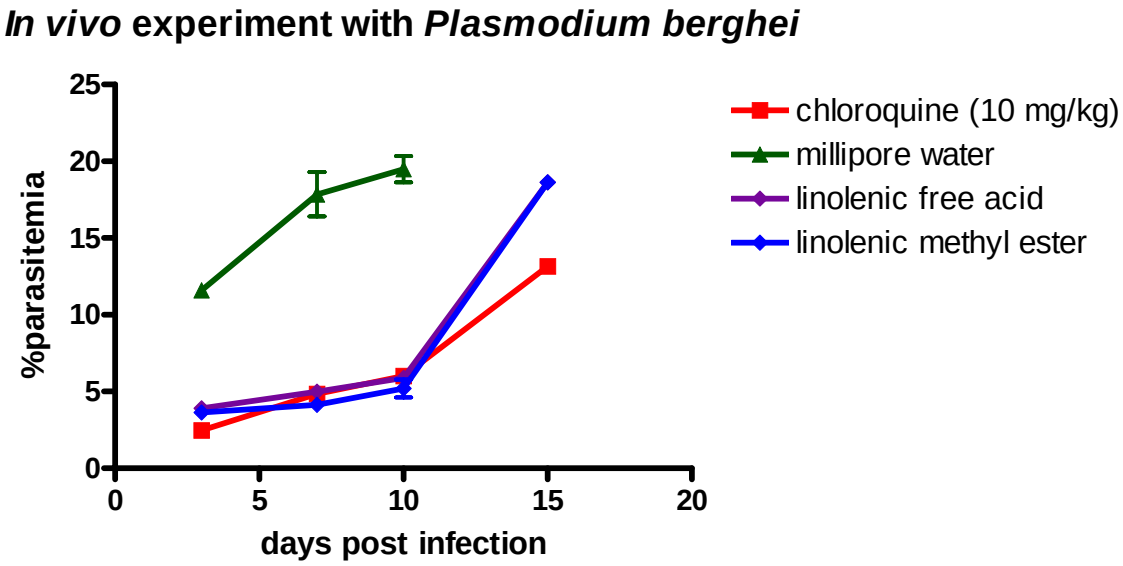

Fig 3.3.2: The *in vivo* activity of linolenic acid and its methyl ester in a 4-day suppressive test using the oral route of administration.

3.4 In vivo schizontocidal experiment using the intravenous route of administration:

linolenic acid methyl ester showed relatively promising activity orally, however, recrudescence occurred spost treatment. It was therefore decided to check the schizontocidal effect of the compound after intravenous administration in order to compare this with the observations using the oral route. A group of mice were infected with *P. berghei* and treated intravenously with linolenic acid methyl ester (100 mg/kg) once a day for four days. The positive control compound is CQ at a dose of 10mg/kg as used previously in the oral route. Results are shown in fig 3.4.1

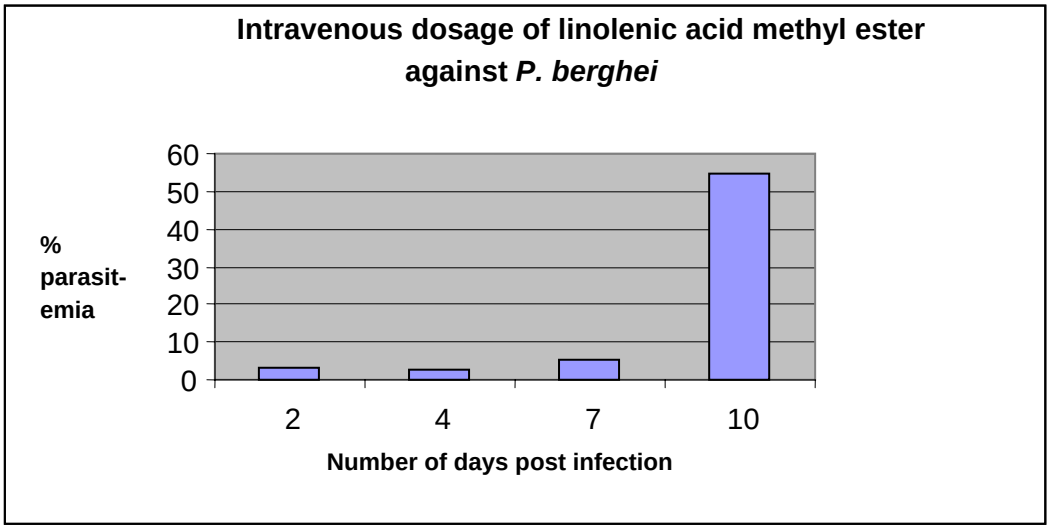

Fig 3.4.1 Percentage parastemia post-infection using a 4-day suppressive treatment against P. berghei.

Parasites were markedly suppressed during the 4-day treatment, but a rapid recrudescence with parasitemia of >50% was recorded on day 10 post infection (Fig 3.4.1).

4. DISCUSSION

The activity of linoleic acid 7.56µg/ml against Dd2 resistant strain in the present study (Table 3.1.1) compares well with the antipalsmodial activity 7.2µg/ml of a naturally occurring C_{18} fatty acid known as *Scleropyrum wallichianum* against the K1 resistant strain [32]. A previous study reported the antiplasmodial activity with

IC50's of 12-16 µg/ml of the very long-chain fatty acids C23-C26 [33]. They also reported that these acids were not toxic and were good inhibitors (IC50's 0.35µg/ml) of the *P.falciparum* enoyl-ACP reductase (FabI) enzyme which is vital to the fatty acid elongation cycle in *P.falciparum*. Kumaratilake *et al*., [4], reported the activity of the unsaturated fatty acids to increase with increase in the degree of unsaturation. The activity of the purchased compounds (99% pure) when tested *in vitro* compares well with the reported activity of linoleic and linolenic acids (<10µg/ml) isolated from the ethyl acetate extract of *C. papaya* in a previous study [34]. The IC50 values do not appear very impressive at a first glance. However, by experience compounds killing cultured malaria parasites at this concentration range can be expected to posses some specific activity which deserves further investigation.

The *in vivo* result from this study demonstrated that linolenic acid was more potent than linoleic acid. This correlates well with the observations of Kumaratilake *et al*. [4] which showed that fatty acids vary in their ability to inhibit the growth of parasites. Their work demonstrated that this variation in fatty acid antiplasmodial activity is partly dependent on the degree of unsaturation. The polyunsaturated compounds they investigated: $C_{22:6,n=3}$, $C_{20:5,n-3}$, $C_{20:4,n-6}$ and $C_{18:2,n-6}$ markedly inhibited the growth of the parasites, while the mono-unsaturated fatty acid $C_{18:1,n-9}$ and the saturated fatty acid C_{22}:0 showed little effect. Kumaratilake *et al.* [4] further reported that the growth inhibition of parasites by the 22-C fatty acid significantly increased several-fold by increasing the degree of unsaturation of the molecule by six. Similarly, when they introduced a single double bond to the mono-unsaturated fatty acid, the antiplasmodial effect of the molecule was more than tripled. These researchers concluded that the polyunsaturated fatty acids were more effective against *P. berghei* in mice when compared to the saturated fatty acids which had no effect.

Fatty acids were reported to cause degeneration in the intra erythrocytic stages of *P. falciparum in vitro* [4]. In a previous study, docosahexaenoic acid [22:6(n-3)] at concentrations of 20-40µg/ml, resulted in the death of 90% of *P. falciparum* [4]. The authors further reported that the fatty acids were not toxic to the red blood cells. In the present study, linolenic and linoleic acids inhibited the growth of parasites by 70% and 64%, respectively on day 4 of treatment. There was marked inhibition of parasite growth by a 1:1 combination of linolenic and linoleic acids. The growth inhibition of compounds linolenic and linoleic acids in combination was 96% on day 4 of treatment. This suggests a possible synergistic effect. After the final dose on day 4, parasite growth remained suppressed until day 10 but by day 15 significant recrudescence was observed for the compounds administered singly, while the result for the mixture suggested that the two compounds were enhancing the activity of each other. It is also possible that the fatty acids used in the present study may have disrupted the fatty acid synthase II pathway of the parasites. Previous studies reported that the biosynthesis of type II fatty acid synthase (FAS II) which takes place in the apicoplast of *P. falciparum* could be altered by fatty acids [2]. The type II FAS II system is vital to these apicomplexan parasites and is also found in

bacteria and algae. The fatty acid biosynthesis of parasites differs from that of humans or higher eukaryotes (type I fatty acid synthase FASI), and this makes it possible for the parasite to be destroyed without harming the host [2]. Previous studies have shown that fatty acid methyl esters are more stable and show good chromatographic properties compared to the free acids [35]. In the present study, it was observed that the *in vivo* antimalarial activities of linolenic and linoleic acids compares well with their methyl esters. These findings were similar to those of Kumaratilake *et al*., [4] who reported similar antiplasmodial activity of a range of free acids and their methyl esters.

Researchers have reported the low levels of 18:3n-3 fatty acids, and further pointed out the differences between their metabolism and that of 18:2n-6 which is usually more abundant [36]. Oxidation of the methyl ester to its active metabolites could explain the *in vivo* activity recorded when this compound was administered orally. Fatty acid methyl esters are metabolized as would be other dietary fats. Higher molecular weight aliphatic esters are readily hydrolyzed to the corresponding alcohol and acid. These are subsequently oxidized to carbon dioxide and water through an established mechanism of metabolic breakdown into two-carbon fragments. These fragments are utilized by the body for energy and serves as building blocks for synthesis. During digestion they are hydrolyzed to the free fatty acids for absorption from the intestine into the blood stream aided by lipase enzymes and bile salts. Once formed, the free acid is metabolized by known oxidative processes or are reconstituted into glyceride esters and stored in the fat depots in the body. Further work by Kumaratilake [37] confirms the oxidation of this class of compounds. The oxidized form of these fatty acids was reported to show increased antimalarial activity [37]. This suggests that the *in vivo* activity recorded when this compound was administered orally could be traceable to the active metabolites. Rapid metabolic conversion of linolenic acid to linoleic acid isomers in the oral administration has been reported by other researchers [11]. A previous study demonstrated that the absorption of conjugated linolenic acid (CLnA) is slow in the rat intestine and is rapidly converted to conjugated linoleic acid [14]. Their report, which was based on the CLA isomers investigated, α-eleostearic acid (9*Z*,11*E*,13*E*-18:3) and punicic acid (9*Z*,11*E*,13*Z*-18:3) showed that after 1 hour of administration, the lymphatic recovery of the conjugated fatty acids with three double bonds was significantly lower compared with the conjugated linoleic acid (CLA) with two double bonds and they recorded that this observation persisted for eight hours [14]. Their study suggested that this difference in absorption of CLA and conjugated triene fatty acids could be due to the rapid conversion of conjugated triene fatty acids to CLA. This, they further explained, was due to Δ^{13}-saturation enzyme reaction which the conjugated triene fatty acids undergo as a result of their slow secretion from the intestine. This slow absorption and rapid conversion to CLA could possibly be responsible for the high rate of recrudescence observed soon after the oral treatment with linolenic acid methyl ester was completed.

This study further investigated the in vivo schizontocidal activity of linolenic acid methyl ester using the intravenous route, since this route could bypass the gastrointestinal metabolism inherent in the oral route. When infected mice were treated intravenously with linolenic acid methyl ester, parasite growth was restricted by 97%, with a 69% recrudescence 3 days post-treatment. The fast recrudescence observed during the *in vivo* antiplasmodial study of this compound intravenously could be attributed to a short half-life, rapid elimination as well as inadequate dose reaching the target site. It has become necessary therefore, to identify drug delivery systems or formulations, which will have mechanisms of action different from the current therapies to address the issues of metabolism, bioavailability and half life of therapeutic agents.
The presence of vitamin E, which is an anti-oxidant, suppresses the antiparasitic activities of this class of compounds [4]. This suggests that diets rich in antioxidants such as vitamin E could counteract the antiplasmodial effect of these fatty acids. This may also indicate that using this class of compounds as antimalarials will require that diets of experimental animals should contain no antioxidants. However, since antioxidants are present in most diet, eliminating them is almost impossible hence fatty acids are not likely to be good antimalarials when used alone. Combination treatments could delay or prevent resistance. EFA when used in combination could delay the onset of resistance, as well as enhance the activity of other compounds in the mixture, since this class of compound seems to have a different mechanism of action when compared to the conventional drugs. Kumaratilake *et al*. [37], further demonstrated the significant structural differences between the fatty acids which induced killing by acting directly on the parasites, and those whose antimalarial activity are products of neutrophil priming. The mechanism of parasite adherence to the neutrophils, and how this can result in the death of parasites, is not known.

5. CONCLUSION AND RECOMMENDATION

The activity of this class of compound is partly influenced by the degree of unsaturation. Chemical modification of this compound by the introduction of additional double bonds could enhance its activity. The oxidized form of these fatty acids showed increased activity. Therefore chemical oxidation of this class of compounds could also enhance efficacy. However, due to early recrudescence recorded in mice after a 4-day suppressive treatment, it is recommended that treatment with this compound be given 2 to 3 times a day and for a longer duration, and possibly be administered in combination with existing antimalarials. Slow release mechanism could be explored using nanodrug delivery systems. The non-mevalonate isoprenoid biosynthesis pathway is also shared by several other pathogens such as *Mycobacterium tuberculosis* and Gram negative bacteria*. This may suggest that innovative methods aimed at targeting* this pathway such as

nanomedicine drug delivery systems could help to deliver broad-spectrum antibacterial, antituberculous, and antimalarial agents.

Nanomedicine has received a great deal of attention in drug delivery and is generally considered as the key technology of the 21st century. It is a multidisciplinary science which could be applied for treating and preventing disease. Nanomedicine based drug delivery systems can lead to improved solubility, oral bioavailablity and highly specific site-targeted delivery of therapeutic compounds. By encapsulating drugs in nanocarriers, they can be protected from degradation and excretion in the intestine, liver and kidneys. Old, current and new therapeutic agents can therefore be redesigned or improved through nanomedicine, leading to improved pharmacokinetics, solubility, reduction in dose and dose frequency and reduced toxicity thus giving them a better chance of being effective and reaching market. These systems have revolutionized therapies for diseases like cancer [38], but have not been widely applied to transform therapies for infectious diseases of poverty such as malaria. We propose the use of nanomedicine and the administration of drugs in combination to address the current shortfalls of malaria therapies. Through this approach, we will be able to increase the availability of the drug at the target area, therefore enabling reduction of the currently high dose and dose frequency, treatment time and hence decrease in toxicity and resistance.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References

[1] G.I. McFadden, M. Reith, J. Munholland, and N. Lang- Unnasch, Plastid in human parasites, Nature, (1996), 381-482.

[2] N.M. Carballeira, New advances in fatty acids as antimalarial, antimicrobial and antifungal agents, Progress in Lipid Research 47 (2008), 50-61.

[3] N.D. Undurti, Can essential fatty acids reduce the burden of disease(s), Lipids in Health and Disease, (2008), 7:9 doi:10.1186/1476-511X-7-9.

[4] L.M. Kumaratilake, B.S. Robinson, A. Ferrante, and A. Poulos, Antimalarial properties of n-3 and n-6 polyunsaturated Fatty acids: *in vitro* effects on *Plasmodium falciparum* and *in vivo* effects on *P. berghei*, Journal of Clinical Investigation, 89 (1992), 961-967.

[5] U.N. Das, Essential fatty acids: Biochemistry, physiology, and pathology, Biotechnology Journal, 1 (2006), 420-439.

[6] U.N. Das, Essential fatty acids-a review, Current pharmaceutical Biotechnology, 7 (2006), 467-482.

[7] T.M. Larsen, S. Toubro, and A. Astrup, Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and humans studies, Journal of Lipid Research 44 (2003), 2234-2241.

[8] J. M. Gaullier, J. Halse, K. Hoye, K. Kristiansen, H. Fagertun, H.Vik, and O. Gudmundsen, Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy overwight humans, Journal of Nutrition, 135 (2005), 778-784.

[9] M. E. Peter, E. B. Barbara, and A.Z. Vincent, The permeability barrier in essential fatty acid deficiency: Evidence for a direct role for linoleic acid in barrier function, The Journal of Investigative Dermatology, 74 (1980), 230-233.

[10] J. Dobryniewski, S.D. Szajda, N. Waszkiewicz, and K. Zwierz, "Biology of essential fatty acids (EFA),".*Przegl Lek*,vol. 64, pp. 91-9, 2007.

[11] T. Tsuzuki, Y. Tokuyama, M. Igarashi, and T. Miyazawa, "Tumor growth suppression by α-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via lipid peroxidation," *Carcinogenesis* vol. 25, pp.1417-1425, 2004.

[12] A. Cesano, S. Visonneau, J.A. Scimeca, D. Kritchevsky, and D. Santoli, Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice Anticancer Research 18 (1998), 1429-1434,.

[13] M.A. Belury, Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action, Annual Review of Nutrition 22 (2002), 505-531.

[14] T. Tsuzuki, Y. Kawakami, R. Abe, K. Nakagawa, K. Koba, J. Imamura, T. Iwata, I. Ikeda, and T. Miyazawa, Conjugated linolenic acid is slowly absorbed in rat intestine, but quickly converted to conjugated linoleic acid, The Journal of Nutrition, Biochemical, Molecular, and Genetic mechanisms v 136 (2006), 2153-2159.

[15] A.K. Mueller, M. Labaied, S.H.I. Kappe, and K. Matuschewski, Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine, Nature, 433 (2005a), 164-167.

[16] M.R. Van Dijk, B. Douradinha, B. Franke-Fayard, V. Heussler, M.W. van Dooren, B. van Schaijk, G. van Gemert, R.W. Sauerwein, M.M. Mota, A.P. Waters, and C. Janse, Genetically attenuated, P36p-deficient malarial sporozoites induce protective immunity and apoptosis of infected liver cells, *PNAS*. 102 (2005), 12194-12199.

[17] R.van D. Melissa, D. Bruno F.F. Blandine , H. Volker, W. van D. Maaike, S. Ben van Jan van G. Geert, W.S. Robert, M.M. Maria, P. W. Andrew and J.J. Chris, "Genetically attenuated, P36p-deficient malarial sporozoites induce protective immunity and apoptosis of infected liver cells," *Proceedings of* National Academy of Science 102 (2005), 12194–12199.

[18] N.V. Thanh, A.F. Cowman, D. Hipgrave, T.B. Kim, B.Q. Phuc, L.D. Cong and B.A. Biggs, Assessment of susceptibility of *Plasmodium falciparum* to chloroquine, quinine, mefloquine, sulfadoxine-pyrimethamine and artemisinin in southern Viet Nam, Transactions of the Royal Society for Tropical Medicine Hygiene 95 (2009), 513-517.

[19] E.J. Scholte, B.G.J. Knols, and W. Takken, Infection of the malaria mosquito Anopheles gambiae with the entomopathogenic fungus Metarhizium anisopliae reduces blood feeding and fecundity, Journal of Invertebrate Pathology 91 (2006), 43-49.

[20] A.O. Talisuna, P. Langi, T. K. Mutabingwa, E. Van Marck, N. Speybroeck, T.G. Egwang, W.W. Watkins, I. M. Hastings, and U. D'Alessandro, Intensity of transmission and spread of gene mutations linked to chloroquine and sulphadoxine-pyrimethamine resistance in falciparum malaria International Journal for Parasitology 33 (2003), 1051-1058.

[21] N. J. White, Drug treatment for falciparum malaria, European Journal of Clinical Pharmacology, 34 (1988), 1–14.

[22] G. A. Balint, Artemisinin and its derivatives: an important new class of antimalarial agents, *Pharmacology and Therapeutics* 90 (2001) 261-265.

[23] T. Gordi, and E. Lepist, Artemisinin derivatives: toxic for laboratory animals, safe for humans?, *Toxicology Letters* 147 (2004), 99–107.

[24] T. J. Anderson, S. Nair, S. Nkhoma, J. T. Williams, M. Imwong, P. Yi, D. Socheat, D. Das, K. Chotivanich, N.P. Day, N.J. White, and A. M Dondorp, High heritability of malaria parasite clearance rate indicates a genetic basis for artemisinin resistance in western Cambodia, Journal of Infectious Diseases, 201 (2010), 1326-1330.

[25] H. Noedl, Y. Se, K. Schaecher, B.L. Smith, D. Socheat, and M.M. Fukuda, Artemisinin Resistance in Cambodia 1 (ARC1) Study Consortium, The New England Journal of Medicine, 359 (2008), 2619-2620.

[26] A. M. Dondorp, F. Nosten, P. Yi, D. Das, A. P. Phyo, J.Tarning, K.M. Lwin, F. Ariey, W. Hanpithakpong, S.J. Lee, P. Ringwald, K. Silamut, M. Imwong, K. Chotivanich, P. Lim, T. Herdman, S.S. An, S. Yeung, P. Singhasivanon, N. P. Day, N. Lindegardh, D. Socheat, and N.J. White, Artemisinin Resistance in *Plasmodium falciparum* Malaria, The New England Journal of Medicine, 361(2009), 455-67.

[27] Medical Research Council (South Africa), Guidelines on ethics for medical research: Use of animals in research and training, Book 3, 2004. http://www.sahealthinfo.org/ethics/book3.htm

[28] W. Trager, and J.B. Jensen, Human malaria parasites in continuous culture, *Science* vol. 193, pp. 673-675, 1976.

[29] M.T. Makler, J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins, and D.J. Hinrichs, Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity, American journal of Tropical Medicine and Hygiene, 48 (1993), 739-741.

[30] W. Peters, B. L. Robinson, G. Tovey, J. C. Rossier, and C. W. Jefford, The chemotherapy of rodent malaria. L. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 3: Observations on Fenzan-50F', a difluorinated 3,3´-spirocyclopentane 1,2,4-trioxane Annals of Tropical Medicine and Parasitology, 87 (1993), 111-123.

[31] L. Tona, K. Mesia, N.P. Ngimbi, B Chrimwami, Okond'ahoka, K. Cimanga, T. de Bruyne, S. Apers, N, Hermans, J. Totte, L. Pieters, and A.J. Vlietinck, *In-vivo* antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and

Phyllanthus niruri, Annals of Tropical Medicine and Parasitology, 95 (2001), 47-57.

[32] A. Suksamrarn, M. Buaprom, S. Udtip, N. Nuntawong, R. Haritakun, and S. Kanokmedhakul, Antimycobacterial and antiplasmodial unsaturated carboxylic acid from the twigs of *Scleropyrum wallichianum*, Chemical and Pharmaceutical Bulletin, 53 (20050, 1327-1329.

[33] N.R. Sperandeo, and R. Brun, Synthesis and biological evaluation of pyrazolylnaphthoquinones as new potential antiprotozoal and cytotoxic agents, A European Journal of Chemical Biology, 4 (2003), 69-72.

[34] P.E. Melariri, W.E., Campbell, P.E. Etusim, and P.J. Smith, Antiplasmodial properties and bio-assay guided fractionation of ethyl acetate extracts from *Carica papaya* leaves Journal of Parasitology Research, In press, 2011.

[35] C. Viron, P.A. Saunois, B. Perly, and M. Lafosse, Isolation and identification of unsaturated fatty acid methyl esters from marine micro-algae Analytica Chimica Acta 409 (2000), 257-266.

[36] R.J. Pawlosky, G. Ward, and Jr, N. Salem, Essential fatty acid uptake and metabolism in the developing rodent brain, Lipids, 31 (1996), 103-107.

[37] L.M. Kumaratilake, A. Ferrante, B.S. Robinson, T. Jaeger, and A. Poulos, Enhancement of Neutrophil-Mediated killing of *Plasmodium falciparum* asexual blood forms by fatty acids: Importance of fatty acid structure, Infection and Immunity, 65 (1997), 4152-4157.

[38] Y. Malam, M. Loizidou, and A. M. Seifalian, Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer, Trends in Pharmacological Sciences, 30 (2009): 592-599.

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