Research Article

Combining Artesunate-Amodiaquine and Ciprofloxacin Improves Serum Lipid Profile of Mice Exposed to *Plasmodium Berghei Berghei*

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Abstract

We investigated the effects of combining artesunate-amodiaquine and ciprofloxacin on the serum lipid profile of mice exposed to *Plasmodium berghei berghei*. The use of this combination for the treatment of febrile illness, suspected malaria and malaria-typhoid coinfection is very common in Nigeria. 60 adult mice and *Plasmodium berghei berghei* were used in this study. Calculated amount of the drugs was administered orally based on dosages of 4mg/kg body weight of Artesunate, 10mg/kg body weight of amodiaquine and 9mg/Kg body weight of ciprofloxacin. The drugs were administered in two divided doses for 3 and 5 days for ACT and ciprofloxacin respectively. The result of our study showed that treatment with the combination of ACT and ciprofloxacin improved serum TG levels better than groups treated with either ACT or ciprofloxacin alone. There were significant ($p<0.05$) increases in the HDL-chol and insignificant ($p>0.05$) changes in VLDL-chol and LDL-chol of groups treated with ACT and ciprofloxacin only in comparison with the normal control. Animals treated with ACT plus ciprofloxacin showed insignificant ($p>0.05$) increases in all fractions of cholesterol tested in comparison to the normal control. All parameters in treated groups tested showed significant increases when compared to the parasitized untreated animals. The result of our study showed that the use of this triple regimen in management of malaria restored the lipid profile deranged by the disease better than either ACT or ciprofloxacin alone. Although synergism in the drugs action may be considered, the actual mechanism is a matter of further research.

Keywords: Combination therapy, ciprofloxacin, malaria, lipid profile

1. Introduction

Combined drug therapy, whether as polyherbal, synthetic agents or both is fast replacing monotherapeutic approaches in the management of many diseases and clinical entities. This is not only due development of resistance by infective agents, but also due to co-existence of disease entities[1,2]. Different combinations and formulations of chemotherapeutic agents have been designed and employed in the treatment of clinical entities, especially in the Sub-Sahara Africa. Information on the biochemical implications of many of these combinations is scarce. The treatment of malaria and development of effective antimalarial drugs have posed great challenge to medicine. This is due to the development of resistance by parasites to most antimalarial agents [3], resulting in immense impact on the socio-economy of man[4,5]. The most important new class of antimalarial agents is the artemisinins, which are natural products developed in China beginning in the 1960s [6]. Antimalarial chemotherapy has been the primary option in the fight against malaria. However, the burden of this disease is still very heavy partly due to the development of multi-drug resistant *Plasmodium falciparum* strains[7-9].

African countries have recently stepped up their antimalarial efforts, and are deploying diverse strategies to contend the new face of malaria. One of these strategies is the use of artemisinin-based combination therapies (ACTs) recommended by WHO, which have proven to be very effective against malaria in sub Saharan Africa, and some African countries plagued with resistant forms of *P.falciparum* have started instituting the ACTs as first line malaria treatment[10,11].

Artesunate plus amodiaquine combination is one Artemisinin–based combination therapy (ACT) recommended by the World Health Organization (WHO) for use in malaria control programmes and a first line treatment for African patients with uncomplicated malaria[11]. It has been suggested that along with antimalarial drugs, other medications that may improve the serum status of the affected biochemical parameters should be incorporated in the treatment strategy during and after malaria infection. This is imperative in view of the immense importance of the serum components affected[12]. Quinolones, particularly ciprofloxacin and pefloxacin, are potent antimalarial drugs which might prove useful in the treatment of less rapidly aggressive human malaria[13].

The present study investigated the effects of combining one of the ACTs (artesunate-amodiaquine) with ciprofloxacin on the serum lipid profile of adult mice exposed to malaria parasites. This triple combination is a very common management modality for febrile illness in Nigeria, where malaria-typhoid co-infection is the commonest diagnosis.

2. Material and Methodology

60 adult mice obtained from Animal House of the College of Health Sciences of the University of Uyo, Nigeria, were randomly distributed into 5 groups. 4 groups were infected with malaria parasites, while 1uninfected group was used as the normal control. The mice weighed between 30 - 35g each. *Plasmodium berghei berghei* were obtained from the Research Unit of the Animal House, University of Uyo, Nigeria

2.1 Inoculation of parasites and treatment of animals

The parasites were inoculated into 2 mice and the level of parasitaemia was assessed after 6 days, by slide preparation using the method described by Greenwood and Armstrong[14]. Parasitaemia level was found to be very high 0n the 6th day. After the achievement of high level of parasitaemia in the mice, blood samples were collected from them and diluted in normal saline at the ratio of 75% parasitized blood and 25% normal saline[12]. The diluted parasitized blood was then inoculated into the appropriate groups of mice via intraperitoneal route. The level of parasitemia was found to be high on the 7th day. However, the mice were allowed to stay for another 3 days making a total duration of 10 days before drug administration. Calculated amount of the

drugs was administered orally based on dosages of 4mg/kg body weight of Artesunate, 10mg/kg body weight of amodiaquine and 9mg/Kg body weight of ciprofloxacin. The drugs were administered for 3 and 5 days respectively for ACT and ciprofloxacin. One of the infected groups was untreated control.

The animals were kept in plastic cages with stainless steel mesh at the bottom that made faeces and feed droppings from the feeding trays inaccessible to the experimental animals. Feeding of rats was *ad libitum*. The animals had access to drinking water from water bottles fitted with stainless steel spout. The animals were fed *ad libitum* with Guinea grower feed and keep at room temperature of 28.0 ± 2^0C.

All the experimental mice (both control and test groups) were sacrificed at the end of treatments and blood samples were collected by thoracotomy and cardiopuncture into appropriately labeled bottles for analysis of HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, total cholesterol, and triglyceride.

2.3 Statistical analysis

All data were expressed as mean ± standard deviation (SD). Analysis of Variance (ANOVA) will be used to analyze data, while Student's t-test was used for comparison. Any difference in mean was considered significant at P<0.05.

3. Results

The results of serum lipid profile of adult mice from analysis of the control group, groups treated with ACT only, ciprofloxacin only and ACT plus ciprofloxacin are shown in Table 1. The results show that animals treated with combination of ACT and ciprofloxacin had a significant (P ≤ 0.05) reduction in the level of total cholesterol when compared to untreated infected group, while the animals treated with either ACT or ciprofloxacin only showed insignificant (p≥0.05) reduction.. Combination of ACT and ciprofloxacin showed total cholesterol level that is almost same with the normal control. Groups treated with either ACT or ciprofloxacin only showed insignificant (p≥0.05) reduction in total cholesterol level when compared to the untreated mice.

There were significant (p≤0.05) increases in the HDL-chol and insignificant (p≥0.05) changes in VLDL-chol and LDL-chol of groups treated with ACT and ciprofloxacin only in comparison with the normal control. Animals treated with ACT plus ciprofloxacin showed insignificant (p≥0.05) increases in all fractions of cholesterol tested in comparison to the normal control. All parameters tested showed significant increases in treated groups in comparison to the parasitized untreated animals.

Treatment with a combination of ACT plus ciprofloxacin improved serum TG levels better than groups treated with either ACT or ciprofloxacin alone

Table 1: Serum Lipid Profile of adult experimental mice treated with ACT, ciprofloxacin and combination of ACT plus ciprofloxacin and untreated control groups.

GROUP[a]	Total Chol. (mmol/l)	Triglycerides (mmol/l)	HDL CHOL (mmol/l)	VLDL CHOL (mmol/l)	LDL CHOL (mmol/l)
Normal Control	3.10±0.25	1.31±0.12	1.25±0.09	1.05±0.13	0.80±0.21
Parasitized Untreated	5.56±0.24*	2.22±0.31*	3.21±0.03*	1.30±0.07	1.06±0.11*
ACT (AM+AS) only	4.17±0.42*∞	1.53±0.20 ∞	2.19±0.11*∞	1.01±0.08	0.98±0.32
Ciprofloxacin only	4.51±0.19*	1.68 ±0.23 ∞	2.65±0.02*∞	0.98±0.05	0.87±0.03
ACT(AM+AS) + Ciprofloxacin	3.42±0.40*∞	1.41±0.53*∞	1.31±0.11*∞	1.22±0.13	0.91±0.26

ACT = artemisinins based combined therapy, AM = amodiaquine, AS = artesunate, a = mean ± SD of 12 determinations.

* = p ≤ 0.05 (Normal control); ∞ = p≤ 0.05 (Parasitized untreated)

5 Discussions

Our results showed a derangement in serum lipid profile of *Plasmodium* infected mice when compared to the normal control group. This finding is consistent with those in other studies that showed elevated levels of serum lipids like HDL, LDL, total cholesterol and triglyceride in patients suffering from malaria infection[12,15,16]. However, Miller *et al*,[17] had earlier reported no significant difference in concentrations of the serum cholesterol between malaria infected and uninfected groups The evidence of higher concentrations of serum lipids in the infected group despite the requirement of lipids for the growth of the parasite, could be explained from the recent findings which suggest that the plasmodium genome contains genes encoding enzymes of phospholipids metabolism, allowing de novo synthesis of phosphatidy choline via the kneddy pathway and necessitating only the uptake of the small choline molecule[12,18,19].

In addition, the genome of the parasite contains genes similar to those for type II fatty acid synthesis pathway. The protein products of these genes are located within the apicoplast and allow for the production of fatty acids, some of which are unique to the parasite[20]. Thus the parasite may be able to meet many of its lipid requirements from its own biosynthetic pathways. The increased in the serum lipid concentrations observed in the malaria infected group has also been attributed to reduced serum concentration of albumin obtained in malaria infection. [12]. Albumin is required to bind to neutral fats (triglyceride, and cholesterol) for these lipids to be transported in the plasma (because the lipids are hydrophobic in nature and therefore requires some forms of hydrophilic adaptation in the form of lipoproteins)[12]. However, infection can lead to a fall in serum albumin due to decreased synthesis of albumin and/or in-creased catabolism of albumin, consequently causes reduction in the binding capacity, and leading to increased plasma free concentration of these lipids.

Our study showed the triple regimen almost normalized serum cholesterol levels to pre-infected state. The significant reduction in the serum total cholesterol in mice treated with ACT plus ciprofloxacin may be due to synergism in eliminating the parasites. Cholesterol is synthesized in the liver [21], which happens to be the major site of plasmodium infection and this raises some questions whether there is any relationship between the cholesterol synthesis by the liver and the plasmodium infection of the liver. It has been postulated that the parasite has ways that enables it to thrive and multiply using nutrients from the host, they still cannot synthesize majority of their own lipids and cholesterol in vivo [12,21]. The fact that the serum cholesterol levels of infected mice is not low when compared with the uninfected group, as one would expect, suggests that the parasites may be synthesizing their needed cholesterol.

Significant increase in serum triglycerides recorded in *Plasmodium* infected animals implicated an unfavourable lipid profile[12,21], which can be deduced that malaria infection can either precipitate or worsen a preexisting cardiovascular disorder.

 Our study showed that treatment of *Plasmodium* infected mice with a combination of artesunate-amodiaquine (ACT) plus ciprofloxacin generally improved serum lipid profile better than groups treated with either ACT or ciprofloxacin alone. A rise in TG has been generally identified as a risk factor for atherosclerotic diseases.

6. Conclusion

The result of our study infers that the use of this triple regimen consisting of artesunate, amodiaquine and ciprofloxacin in management of malaria restored the lipid profile deranged by the disease better than either the ACT or ciprofloxacin alone. The effect may be due to synergism in the drugs action, though this is considered a subject for further studies.

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