Effect of artesunate and amodiaquine alone and in combination on plasma biochemical parameters in mice

Abstract
Albino mice are considered a comparable genetic model to humans and it is well established that they also exhibit natural differences in susceptibility to malaria infection. The study was aimed at determining and comparing the effects of artesunate, artesunate+amodiaquine combination, on biochemical parameters such as plasma pH, plasma glucose and plasma cholesterol in the course of administering antimalarial drugs. The effects of artesunate, amodiaquine and a combination of artesunate-amodiaquine on some hematological and biochemical parameters were assessed in this study. Twenty albino mice of eight weeks old were randomly divided into 4 groups based on a specific antimalarial drug administered and one group served as control. Blood sample was obtained at the end of the study and assay was done for glucose concentration, plasma pH, and plasma cholesterol concentrations. Data were expressed as mean±standard errors of mean. Comparisons between control and treated groups of albino mice were performed with one-way analysis of variance (ANOVA), followed by Tukey Kramer post hoc test for multiple comparisons. Statistical significance was set at P<0.05. Plasma pH was not significantly lower (p>0.05) in the antimalarials; artesunate, amodiaquine and artesunate+amodiaquine groups compared to the control group. Plasma glucose was significantly lower in the antimalarials; Artesunate, artesunate+amodiaquine but higher in Amodiaquine compared to the control group. Plasma cholesterol was significantly lower (p<0.05) in the treated groups, Amodiaquine, artesunate+amodiaquine groups compared to the control group. Hence, maximum reduction was seen in the combination group compared to the individual drugs.

Keywords: amodiaquine, antimalarials, artesunate, albino mice, plasma cholesterol

Introduction
Malarial infection still stands out as one of the widespread parasitic diseases affecting mainly the third world countries yet causing a high morbidity and mortality.1 The parasite has a unique mechanism of action through its invasion of red blood cells, liver and in some severe forms splenic and cerebral involvements.2,3 The complications resulting from malaria could be in the acute or chronic phase mediated by direct parasitic invasion of tissues or distortions from altered biochemical processes.4,5 The role of antimalarials in reducing morbidity and mortality resulting from malaria cannot be understated, especially in sub-Saharan Africa. However, the targets of most health bodies in mitigating the problem have not been met owing to the socio-economic factors affecting healthcare.6 These factors are directly related to the endemicity of destitution in Africa. Erstwhile, malaria’s morbidity and mortality rate were soaring owing to inefficient medications and irrational use of available medications which invariably led to high resistant strains of the *Plasmodium falciparum*.7 Chloroquine-based drugs faced out after the introduction of the artemisinin-based combination therapies (ACTs) which proved effective, safe and economically sound8 but currently remains in use in treating some autoimmune diseases such as discoid lupus erythematosus.9 Amodiaquine, a 4-aminoquinoline similar to chloroquine is used in the treatment of malaria currently in combination with the artemisinin, especially artesunate.10 It acts by an intracellular mechanism halting and shutting down the DNA and RNA machinery.11 Although, well tolerated compared to chloroquine, it results in several haematological and biochemical aberrations such as neutropenia and raised serum transferases.12,13 Artesunate is an artemisinin derivative,14 which is usually used solely or in various combinations. It has a wide therapeutic window,15 leading to its widespread use in malaria. The malarial parasites have no resistance to the artemisinin as at now.16 Notwithstanding, it has shortfalls such as increased rate of recrudescence owing to its short half-life17 hence its sole administration can lead to resistance in future. Combination therapies are usually preferred to avert this unfavorable outcome.18 Measurement of resistance is very difficult and usually recognized at a very latter stage, hence measures need to be put in place to avoid this. Artemisinin and its congeners can produce carbon-based radicals which can cause oxidative stress to surrounding tissues.19 However, the effect of artemisinin compounds and their combinations on various plasma biochemical parameters have neither been evaluated nor compared to some of the older drugs.

Methods
Twenty imprint control (ICR) albino mice of both sexes (25-30g) were obtained and housed in a cage at ambient temperature. The albino mice were fed daily on experimental basis. Study was conducted in accordance with established guidelines for care and use of animals in research by the National Institute of Health (NIH).

Experimental design
The twenty-imprint control (ICR) albino mice were divided into four groups (n=5). Treatment is provided in all groups two times daily at therapeutic doses calculated on weight of the albino mice.

Group A: albino mice were treated with normal saline and served as a control group Group B: albino mice were given 1mg of Artesunate...
Effect of artesunate and amodiaquine alone and in combination on plasma biochemical parameters in mice

**Statistical analysis**

Data was presented in mean±SEM and was analyzed using GraphPad Prism for Windows Version 6.01 (GraphPad Prism Software, San Diego, USA). Results were presented as Mean values±standard error of mean (SEM), and the statistical differences between treatment groups compared using One–way analysis of variance (ANOVA) followed by Tukey Kramer post hoc test for multiple comparisons, with a 95% confidence interval. P<0.05 was considered statistically significant.

**Results**

Plasma pH concentration was reduced in all treatment regimen when compared to the control group. When compared with the saline control group 7.35±0.05, treatment with Artesunate, Amodiaquine and combination of Artesunate+Amodiaquine reduced plasma pH concentration to 7.36±0.01, 7.34±0.01 and 7.40±0.01 respectively (Table 1). None of the drugs resulted in a pH significantly different from the naive control (p>0.05).

**Table 1** Effects of medications on plasma pH

| Treatment                  | pH     | P-value |
|----------------------------|--------|---------|
| Saline Control             | 7.35±0.05 |        |
| Artesunate                 | 7.36±0.02 | 0.09   |
| Amodiaquine                | 7.34±0.01 | 0.08   |
| Artesunate+Amodiaquine     | 7.40±0.01 | 0.197  |

**Table 2** Effects of medications on plasma glucose (g/dL)

| Treatment                  | Glucose | P-value |
|----------------------------|---------|---------|
| Saline control             | 35.58±0.60 |        |
| Artesunate                 | 31.81±0.31 | 0.04   |
| Amodiaquine                | 37.06±0.36 | <0.001 |
| Artesunate+Amodiaquine     | 30.91±0.17 | 0.02   |

**Table 3** Effects of medications on plasma cholesterol

| Treatment                  | Cholesterol | P-value |
|----------------------------|-------------|---------|
| Saline control             | 51.86±0.16 |        |
| Artesunate                 | 39.45±0.09 | <0.001 |
| Amodiaquine                | 43.21±0.78 | <0.001 |
| Artesunate+Amodiaquine     | 32.10±0.52 | <0.001 |

Treatment with Artesunate, Amodiaquine and combination of Artesunate+Amodiaquine therapy at the stated doses significantly reduced plasma glucose concentration to 31.81±0.31, 37.06±0.36 and 30.91±0.17 respectively when compared with 35.58±0.60 in the saline control albino mice (Table 2). All three drugs resulted in variable plasma glucose which was significantly different from the control (p<0.05).

Plasma cholesterol level was elevated to 51.86±0.16 in the saline control group compared to treatment with AR, AM and combination of AR+AM of 39.45±0.09, 43.21±0.78 and 32.10±0.52 respectively (Table 3). Amodiaquine decreased total cholesterol concentration to levels significantly different from the control group.

**Conclusion**

In this present study, the maximum reduction was seen in the combination group compared to the individual drugs. Following was the treatment with Artesunate which was found to reduce plasma glucose significantly. The introduction of the new agents in replacing previously used antimalarial drugs such as quinine and chloroquine was successful. Untoward effects from cinchona alkaloids which was mainly hypoglycemia and allergic reactions were averted with the
introduction of artesunate, amodiaquine and its congeners. In depth toxicological studies of these agents in humans are lacking owing to the spurious perception of these agents having any untoward effects. This study, however, sets the premise for further research with respect to antimalarials in human subjects and focusing on chronic exposure as compared to acute exposure in this study.

Acknowledgments

The authors are thankful to the Department of Applied Chemistry and Biochemistry C.K, Tedam University of Technology and Applied Sciences, Ghana for the use of their laboratory facilities during the research.

Conflicts of interest

The authors declare that this article content has no conflict of interest.

References

1. Murphy SC, Breman JG. Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. The American Journal of Tropical Medicine and Hygiene. 2001;64(1-2 Suppl):57–67.
2. Kerb R, Fax R, Mörike K, et al. Pharmacogenetics of antimalarial drugs: effect on metabolism and transport. The Lancet Infectious Diseases. 2009;9(12):760–774.
3. Klonis N, Creek DJ, Tilley L. Iron and heme metabolism in Plasmodium falciparum. The American Journal of Tropical Medicine and Hygiene. 2007;77(1):14–15.
4. Markham LN, Giostra E, Hadengue A, et al. Emergency liver transplantation in amodiaquine-induced fulminant hepatitis. The American Journal of Tropical Medicine and Hygiene. 2005;72(6):22–27.
5. Zachary Y. Attitude of Mothers towards Complications of Malaria Case of Children Less than 5years Living in New Bell Ebolowa (Cameroon). Malaria Control & Elimination. 2016;5(2).
6. Guidelines for the Treatment of Malaria. World Health Organization: Geneva, Switzerland; 2010.
7. Snow RW. Global malaria eradication and the importance of Plasmodium falciparum epidemiology in Africa. BMC Medicine. 2015;13(1):23.
8. Itoh M, do Valle SN, Farias S, et al. Efficacy of Arteether–Lumefantrine for Uncomplicated Plasmodium falciparum Malaria in Cruzeiro do Sul, Brazil. 2016. The American Journal of Tropical Medicine and Hygiene. 2018;99(1):88–94.
9. Laurentius A Pramono, Suryo Anggoro, Sandra Langow, et al. Successful Treatment of Dissected Lupus Erythematosus with Chloroquine. Acta Med Indones. 2013;45(4):321–323.
10. Famin O, Ginsburg H. Differential effects of 4-aminquinolone-containing antimalarial drugs on hemoglobin digestion in Plasmodium falciparum-infected erythrocytes. Biochemical Pharmacology. 2002;63(3):393–398.
11. Rosenblatt JE. Antiparasitic agents. Mayo Clinic Proceedings: Elsevier; 1992. 276–267 p.
12. Farombi EO. Influence of amodiaquine treatment on microsomal lipid peroxidation and antioxidant defense systems of rats. Basic & Clinical Pharmacology & Toxicology. 2000;87(6):249–254.
13. Yeka A, Lameyre V, Afizi K, et al. Efficacy and safety of fixed-dose artesunate-amodiaquine vs. arteether-lumefantrine for repeated treatment of uncomplicated malaria in Ugandan children. PLoS One. 2014;9(12):e113311.
14. Olurishe TO, Kwanashie HO, Anuka J, et al. Histopathological effects of sub-chronic lamivudine-artesunate co-administration on the liver of diseased adult Wistar rats. North American Journal of Medical Sciences. 2011;3(7):325–328.
15. Li Q, Xie LH, Johnson TO, et al. Toxicity evaluation of artesunate and artelinate in Plasmodium berghei-infected and uninfected rats. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;101(2):104–112.
16. Saifi MA, Beg T, Harrath AH, et al. Antimalarial drugs: Mode of action and status of resistance. African Journal of Pharmacy and Pharmacology. 2013;7(5):148–156.
17. LaCrue AN, Scheel M, Kennedy K, et al. Effects of artesunate on parasite recrudescence and dormancy in the rodent malaria model Plasmodium vinckei. PLoS One. 2011;6(10):e26689.
18. White N. Delaying antimalarial drug resistance with combination chemotherapy. Parasitologia. 1999;41(1-3):301–308.
19. Kavishe RA, Koenderink JB, Alifrangis M. Oxidative stress in malaria and artemisinin combination therapy: Pros and Cons. The FEBS Journal. 2017;284(16):2579–2591.
20. Ayogu E, Ugwuowo O, Amorha K, et al. Evaluation of ciprofloxacin effect on the antimalarial activity of some antimalarial drugs in plasmodium berghei infected mice. International Journal of Pharmaceutical Sciences and Research. 2016;7(5):1896–1903.
21. Onaloapo AY, Onaloapo OJ, Awe EO, et al. Oral Amodiaquine, Artesunate and Artesunate Amodiaquine Combination Affects Open Field Behaviors and Spatial Memory in Healthy Swiss Mice. Journal of Behavioral and Brain Science. 2013;3(8):569–575.
22. Adevyee G, Nneli R, Nwozor C, et al. Effects of Coartem and Artesunate on Some Haematological and Biochemical Parameters in Albino Rats. African Journal of Biomedical Research. 2012;15(1):55–58.
23. Gomes AP, Vitorino RR, Costa AdP, et al. Severe Plasmodium falciparum malaria. Revista Brasileira de Terapia Intensiva. 2011;23(3):358–369.